GAVIN PUBLISHERS

Food & Nutrition Journal

El-Dawayati MM, et al. Food Nutr J 2: 133.
DOI: 10.29011/2575-7091.100033

Research Article

Effect of Different Conservation Periods with Different Sucrose Concentrations on Conserving Somatic Embryos Clusters of Date Palm (*Phoenix dactylifera* L.) Under Minimal Growth Conditions

Maiada M El-Dawayati*, Mohamed A Abdel baki, Lobna M Abdelgalil

The Central Laboratory of Date Palm Researches and Development, Agriculture Research Center, Giza, Egypt

***Corresponding author:** Maiada M El-Dawayati, The Central Laboratory of Date Palm Researches and Development, Agriculture Research Center, Giza, Egypt. E-mail: maiada_dw@hotmail.com

Citation: El-Dawayati MM, Abdel baki MA, Abdelgalil LM (2017) Effect of Different Conservation Periods with Different Sucrose Concentrations on Conserving Somatic Embryos Clusters of Date Palm (*Phoenix dactylifera* L.) Under Minimal Growth Conditions. Food Nutr J 2: 133. DOI: 10.29011/2575-7091.100033

Received Date: 26 May, 2017; **Accepted Date:** 15 July, 2017; **Published Date:** 21 July, 2017

Abstract

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 economical precious tree. The present study was conducted to investigate the possibility of using in vitro slow growth storage for date palm germplasm conservation to promote germplasm exchange and rapid propagation when necessary. Multiply somatic embryos cluster of date palm cv. sukarry were used as conserved explants. Different conservation period 4, 8, and 12 months with different sucrose concentrations at (30,60, 90 or120 g/L) supplemented in conservation medium which consist of 1/2 strength of basal salts of Murashig and Skoog (MS) medium, 30g/L mannitol, 0.05mg/L (BA) benzyladenin,.0.1 mg/L (NAA) Naphthalene Acetic Acid and 8g/L agar, under low temperature at 15◦C and dark of incubation conditions, were studied on the survival and re-growth capacity of date palm conserved explants after returning to the normal growth conditions. Contents of Total Soluble Sugar (TSS), Non-Reducing Sugar (NRS) and Reducing Sugar (RS) were also determined as physiological changes during conservation periods. Results showed that conservation medium supplemented with sucrose at 90 or120 g/L gave the highest significant value of survival percentage respectively after 12 month of conservation period. Best recovery performance under normal growth conditions for conserved somatic embryos cluster under studied minimal growth conditions was achieved when sucrose at 90g/L was used in conservation medium for 8months.

Keywords: Germplasm Conservation; *In Vitro*; Low Temperature; (*Phoenix dactylifera* L.); Slow Growth Storage; Sucrose

Introduction

The date palm (*Phoenix dactylifera* L.) is one of oldest cultivated plants of human kind and used as food for 6000 years [1]. There are more than two hundred varieties [2] of dates available worldwide. Date palm is the most common fruit tree grown in semiarid and arid- regions it plays an important role in the protection of interplant cropping systems and the stabilization of the ecological system [3]. 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 both agriculture and horticulture "Either" for the current production of new plants or preservation of plant genetic resources in order to face the increasing depletion of natural resources [4,5]. *In vitro* propagation of date palm achieved great goal over conventional methods of propagation, through intensive studies and well applied results using techniques such as somatic embryogenesis and organogenesis [6-15].

Germplasm is sum total of all the genes present in a crop and its related species [16]. Date palm germplasm is valuable because it contains diversity of genotypes which needed to be maintained and improved for endangered, elite and commercial varieties. Advances in biotechnological research have opened new avenues for *in vitro* conservation of cultures which has been applied with varying degrees of success to wide range of species and culture sys-

tems by cryopreservation or slow growth procedures depending on the storage duration required [17,18,19].

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 cultivation as genotypes can be effectively conserved without the loss of viability in the form of disease-free stocks in a controlled environment [20]. 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 [20]. 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 [21,22]. Reducing the growth and increasing the storage life by the addition of osmotic agents as sucrose, sorbitol, ribose and mannitol to culture have been proved to be efficient [23]. Osmotic agents act as a growth retardant 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 [24,25]. In general sugar solutions can produce an appropriate osmotic potential [26]. Osmotic potential is generated differently depending on the plant type; therefore, finding the appropriate concentration of the osmotic is needed in order to identify the optimum conditions for *in vitro* short-term preservation. In the present study, the *in vitro* conservation of Arabian cultivar of date palm Sukarry which well established under Egyptian climatic and possesses high fruits quality, was conducted for the first time. Thus, our study could have a positive economical outcome by promoting germplasm exchange and rapid propagation when necessary. We tested the effect of sucrose as osmotic agent added to conservation medium at different concentrations (30, 60, 90, or120g/l) combine 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 each conservation period for three consecutive subcultures during recovery under standard normal growth conditions were studied, by estimating some growth parameters for conserved somatic embryos conversion after preservation, as browning degree, proliferated shoots number, proliferated shoots length, growth vigor. Contents of Total Soluble Sugar (TSS), Non-Reducing Sugar (NRS) and Reducing Sugar (RS) were also determined to discuss physiological changes in sugar metabolism during conservation periods. That exposure of plants to low temperatures induces biochemical and physiological changes which allow them to withstand this stress [27,28]. The aim of the investigation was to devise a conservation technique that is easy to establish, is cost-effective, and provide the maximum regeneration rate for stored cultures. To our knowledge, in this concerns only some studies have been conducted on preserving date palm cultivars by slow growth technique [21,22,25,29-34] but it is still needs more works to determine suitable conservation protocols for elite and important date palm cultivars.

Material and Methods

For obtaining somatic embryos clusters explants which serve as explants material for this experiment: -

Date palm micro propagation by direct somatic embryogenesis

Off shoot of female date palm adult tree of Arbian cultivar Sukkary about (3-5 Kg weight) was taken from healthy mother plant, all outer leaves and sheath were gradually removed carefully tell reached to inner shoot tip about (6-8 cm length) and (3-4 cm width), shoot apices were taken and 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 surfaces sterilization with 0.1 mg/L mercuric chloride ($HgCl_2$) for10 min, then thoroughly rinsed with sterilized distilled water and again with mercuric chloride ($HgCl_2$) for another 50 min, and thoroughly rinse with sterilized distilled water for three times.

Sterilized shoot tips explants were divided into four equals longitudinally sections then cultured on Murashig and Skoog [35] MS nutrient medium supplemented with1.0 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 glutamin, 50g/L sugar, 4mg/L thiamine HCl and 3 g/L Activated Charcoal (AC) (MS1). Cultures were transfer 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 glutamin, 50g/L sugar and 4mg/L thiamine HCl (MS2), cultures were incubated under complete darkness at 27 ± 1˚C.

Direct adventitious buds and somatic embryogenesis were obtained according to [15]. Received multiplied direct somatic embryos clusters (secondary embryos which serve as explants material) Figure 1 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 4mg/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 of all media was adjusted to 5.8, prior to autoclaving at 121˚C for 20 min.

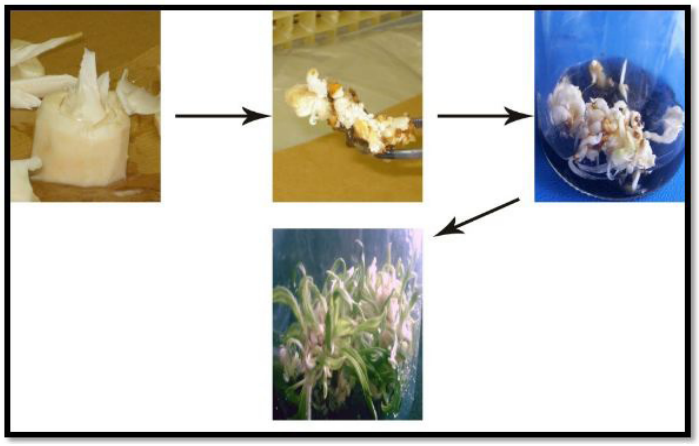

Figure 1: (A) Sterilized shoot tip explants (B), (C) Induction for direct somatic embryogenesis on (MS1) and (MS2) medium, (D) Received multiplied direct somatic embryos clusters (secondary embryos which serve as explants material) on (MS3) medium.

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

Somatic embryo cluster about (8-10 embryos) Figure 2 Were obtained as previously mentioned above (because individually separated somatic embryos were incapable of proliferating further or germinating). Clusters of somatic embryos were placed on conservation medium consist of 1/2 strength salts of MS medium, 0.05mg/L BA, 0.1 mg/L NAA, 30 g/L mannitol and different sucrose concentrations at (30,60, 90 or120 g/L) were added as different treatments. The pH of all conservation media treatments was 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 121oC for 20 min. The culture jars of each treatment were divided into three groups according to the three-tested conservation period (4, 8, and 12). All culture jars were conserved at 15oC under complete darkness.

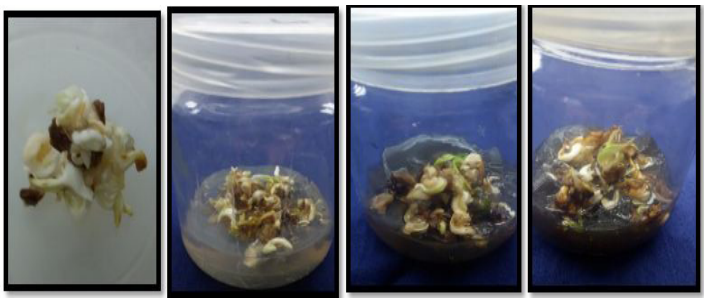

Figure 2: Excessed somatic embryo cluster about (8-10 embryos) as Conserved explants.

Recovery growth conditions of conserved somatic embryos clusters

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

Survival percentage of conserved explants after each conservation period of different treatments was determined after returning the conserved explants on recovery medium under normal growth conditions for 4 weeks. After each conservation period regrowth capacity of conserved explants were taken after three subcultures with (six weeks) intervals on recovery medium under normal growth conditions about browning degree/ explant, number and shoot length/ explant of proliferated shoots from conserved somatic embryos clusters and their growth vigor/explant. Browning degree and growth vigor data were scored visually according to Pottino (1981) [36] as follows: -

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

Biochemical analysis: The changes in Total Soluble Sugar Content (TSS), Reducing Sugar Content (RS) and Non-Reducing Sugar Content (NRS) of conserved somatic embryos explants were recorded at the end of each conservation period (4,8 and 12) for all treatments under studied slow growth conditions.

Determination of the total soluble sugar and reducing sugar

One gram of fresh sample was ground in mortar with 20 ml ethanol 80%, and heated at 70°C for 1 h in water bath for three times. The combined extracts were filtered and evaporated till dryness in water bath at 55 C°. Dried film was dissolved in 10ml of 10% aqueous isopropanol. The aqueous isopropanol extract divided to two sections. The first was taken to determine total soluble sugar and the other to determine the reducing sugar. Total soluble sugars were determined in isopropanol extract by using the phenol - sulphuric method according to A.O.A.C. (1980) [37]. Reducing sugars were determined in ethanolic extract, using phosphomolybdic method according to Dubois, et al. (1956) [38].

Layout of the Experiments

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) [39].

Results and Discussion

In order to determine optimum conditions for inducing minimal growth storage for somatic embryos clusters of date palm cv. Sukarry, our study was designed to study the effect of different sucrose concentrations (30, 60, 90 or 120 g/L) as osmotic agents supplemented in conservation medium. It is worth mentioning that mannitol at low concentration 30 g/L was added with each tested concentration of sucrose to increase the effective 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 and protects plant tissues against oxidative stress damage [40,20], low concentrations resulted in improved survival of stored cultures [41].

The regeneration response of the conserved somatic embryos 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

On recovery medium under normal growth conditions all cultures exposed to different levels of sucrose survived for 4 months, as survival percentage was 100% Table 1 and. It is clearly presented from data that survival percentage of conserved somatic embryos clusters decreased significantly with the increased of conservation period. This result was cope with other studies in slow-growth cultures of date palm [32,34]. Manipulation of sucrose concentrations in conservation medium showed the lowest significant value of survival percentage of conserved somatic embryos clusters cultured on conservation medium supplemented with 30g/L sucrose (62.67) where, at 90g/L of sucrose concentration supplemented in conservation medium the highest significant value of survival percentage of conserved somatic embryos clusters was achieved, following by the survival percentage of conserved somatic embryos clusters on conservation medium supplemented with 120g/L sucrose (85.62, 79.25 respectively) without significant differences in between. *In vitro*, slow-growth storage was efficiently used for mid-term conservation of elite clones of *Chlorophytum borivilianum Sant*. et Fernand when sucrose concentrations at 120g/L which enabled 100% survival from cultures stored for 4 months without any subculture or medium addition [42]. It could be suggested that at high concentrations of sucrose at 90g/L or at 120g/L in conservation medium, conserved somatic embryos clusters cultures grew very slowly; hence, the medium did not get consumed up to12 months. The role of sucrose as osmotic agents at higher concentration was investigated in view of slow-growth conservation studies George (1996) [43] 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 [44]. Panis, et al. (1996) [45] reported that sucrose is responsible for lack of moisture in banana, and because of desiccation sensitivity, *in vitro* developed shoots cannot grow well in media supplemented with higher levels of sucrose. Sucrose was efficiently employed for osmotic stability in potato [46]. The addition of osmotic to culture has been proved to be efficient in reducing growth and increasing the storage life of many *in vitro* grown tissues of different plant species [23]. According to the high levels of osmotic agents in the medium would inhibit both callus growth and shoot formation [47] 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 Sukarry cv for 8 months at highest significant result of survival percentage after slow growth conditions (88.29) Figure 3. On 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.

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

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

Figure 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.

Browning appearance during recovery conditions

Browning appearance of regenerated conserved somatic embryos 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.Where there was no significant differences between the browning degree of regenerated conserved somatic embryos clusters under normal growth conditions for three subcultures after conservation periods for 8 months or 12 months (3.99 ,4.08respectively) . It could be noted from data that regardless of the conservation period sucrose addition into conservation medium at 90 g/L recorded the lowest significant result for browning appearance when conserved somatic embryos clusters returned to normal recovery conditions after conservation period (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 embryos 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 agreement with [48] in African violet, [31] in date palm and [49] in Wild Crocus. From our observation, also low temperature at 15°C of minimal growth conditions had an effective rule in the browning appearance of regenerated conserved somatic embryos clusters under normal growth conditions for three subcultures after conservation periods in this concern (Gianní and Sottile 2015) [50] 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.

Sucrose concentrations g/L(A)	**Conservation period(Months) (B)**			Mean (A)
	4	8	12	
30	1.88^{e}	4.44a^{b}	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}	3.33^{ab}	4.55^{c}	3.55^{a}
Mean (B)	2.39^{b}	3.99^{a}	4.08^{a}	

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

Number of proliferated shoots per explant during recovery conditions

Conserved somatic embryos clusters of date palm cv sukarry 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 embryos 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 embryos 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 subcultures. This result is on line with Tyagi, et al. [26] who found that high sucrose (9%) in culture medium was supportive for induction of *in vitro* rhizomes in *Zingiber officinales*, 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 subcultures. For the interaction effect between sucrose concentration and conservation period on shoots number which converted from the conserved somatic embryos clusters of date palm cv Sukarry 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 embryos 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, 28.37,27.81 respectively).

Sucrose concentrations	**Conservation period (Months)**			**Mean (A)**
mg/L(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}	

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

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 embryos clusters of date palm Sukarry 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 5 somatic embryos 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, 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 embryos clusters under normal growth conditions for three subcultures after conservations periods (3.50, 3.78 respectively) without significant differences in between. Data demonstrated also that somatic embryos clusters of date palm Sukarry 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, 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 subcultures after each studied conservation period (4, 8, and 12 months) without significant differences among them (6.33, 6.83, 6.17 respectively).

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}	

Table 4: Length of proliferated shoots per explant during recovery conditions of conserved somatic embryo cluster of date palm Sukarry cv after (4,8 and 12 months) storage period 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}	

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

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 embryos clusters of date palm Sukarry cv. when returned to resume their developing under normal growth condition for three sub cultures 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 embryos 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, 3.48 respectively without significant differences in between) Figure 4 on opposite the visually rating score for growth vigor of conserved somatic embryos clusters of date palm Sukarry cv under normal growth conditions for three subculture was significantly declined when somatic embryos clusters were conserved on conservation medium with the addition of sucrose at low concentrations at 30 g/L and 60 g/L (3.66, 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 Figure 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 ,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 embryos clusters under normal growth conditions for three subcultures showed green, strong and well-developed shoots after each studied conservation period (4, 8, and 12 months) (4.11, 3.89, 3.66 respectively without significant differences among them).

Figure 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) [51] found that with 90% sucrose was more effective, on conserving tow species of lilly which had been conserved on the original medium for more than 15 months. The tube seedlings conserved for 15 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) [31] reported that explant growth in the presence of sucrose depends on its concentration. Survival and re-growth of the date palm callus decreased significantly as the concentration of sucrose increased in the medium. Elbahr, et al. (2106) [31] 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 [20].

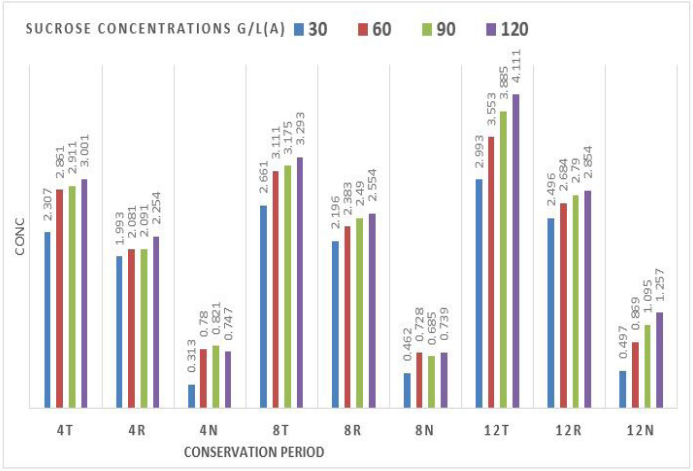

Figure 5: Detected total soluble sugar, reduced sugar and non-reduced sugar contents of conserved somatic embryos of date palm Sukarry cv. on conservation medium supplemented with different sucrose concentrations after different conservation period (4, 8, 12 months) at 15˚C.

Analysis of total soluble sugar, reduced sugar and non-reduced sugar

From data in Figure 5 which determined the Changes in Total Soluble Sugar (TSS), Reducing Sugar (RS) and Non-Reducing Sugar (NRS), in conserved somatic embryos clusters of date palm Sukarry cv. Revealed clearly that the analysis of Total Soluble Sugar (TSS), Reduced Sugar (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. 12 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 embryos 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 embryos 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 Figure 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 embryos clusters were achieved. These values are decreased significantly in ascending order with the decreased in the sucrose concentration to record the lowest

value of (TSS) (2.654), (RS) (2.228) and (NRS) (0. 930) contents of conserved somatic embryos clusters on conservation medium supplemented with sucrose concentration at 30g/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) [22] 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 [52,53]. Kaur, et al. (2012) [54] reported that slow growth was associated with changes in sugar metabolism, they found that changes in starch, Total Soluble Sugar (TSS), Non-Reducing Sugar (NRS), and Reducing Sugar (RS) in *Dendrocalamus hamiltonii* somatic 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) [55] 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 micro propagated plants. It may favor acclimatization and accelerate physiological adaptations [56]. Proline, total sugar, reduced sugar 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 [57]. It could be suggested that when total and reduced sugar 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.

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 Sukarry 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 Figure 6. High sucrose concentration may help to overcome the adverse effects of low temperature during storage period on overall tissue survival and recovery potential. More studies are needed for Date palm germplasm conservation for all important varieties needs extensive studies to seek the optimal protocols.

Figure 6: 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 Figure 6.

References

1. Sulieman A, Elhafise I, Abdelrahim A (2012) Comparative study on five Sudanese date (*Phoenix dactylifera* L.) fruit cultivars. Food Nut Sci 3: 1245-1251.
2. Amer WM (1994) Taxonomic and Documentary Study of Food Plants in Ancient Egypt. Ph.D. Thesis, Cairo University, Egypt.
3. Hasnaoui AM, Elhoumaizi A, Hakkou A, Wathelet B, Sindic M (2011) Physico-chemical Characterization, Classification and Quality Evaluation of Date Palm Fruits of some Moroccan Cultivars. J Sci Res 3: 139-149.
4. Dodds JH (1991) *In vitro* Methods for Conservation of Plant Genetic Resources. Chapman & Hall, London. Pg No: 93-110.
5. Jain M S (2012) Date palm biotechnology: Current status and prospective - an overview. Emirati Journal of Food Agriculture 24: 386-399.
6. Tisserat BH (1979) Propagation of date palm (*Phoenix dactylifera* L.) *in vitro*. J Exp Bot 30: 1275-1283.
7. Sharma DR, Sunita D, Chowdhury JR (1984) Somatic embryogenesis and plant regeneration in date palm *Phoenix dactylifera* L. cv. 437 Khadravi through tissue culture. Indian J Exp Biol 22: 596- 598.
8. Eshraghi P, Zaghami R, Mirabdulbaghi M (2005) Somatic embryogenesis in two Iranian date palm cultivars. Afr J Biotechnol 4: 1309-1312.
9. Othmani A, Bayoudh C, Drira N, Marrakchi M, Trifi M (2009) Somatic embryogenesis and plant regeneration in date palm *Phoenix dactylifera* L. cv. Boufeggous is significantly improved by fine chopping and partial desiccation of embryogenic callus. Plant CellTiss Organ 97: 71-79.
10. Fki L, Masmoudi R, Kriaâ W, Mahjoub A, Sghaier B, et al. (2011) Date palm micropropagation via somatic embryogenesis. In: Jain SM, Al-Khayri JM, Johnson DV (eds) Date Palm Biotechnology. Springer, Netherlands. Pg No: 47.
11. Sidky R, El-dawyati MM (2012) Proliferation of female inflorescence explants of date palm. Ann AgricSci 57: 161-165.
12. Bekheet SA (2013) Direct organogenesis of date palm (*Phoenix dactylifera* L.) for propagation of true-to-type plants. Sci Agr 4: 85-92.
13. Zayed Z (2014) Effect of different types of cytokinins on the regeneration ability of direct somatic embryos and adventitious shoots induced immature inflorescences of date palm. Egypt J of Appl Sci 29: 142-153.

14. Jazinizadeh E, Zarghami R, Majd A, Iranbakhsh A, Tajaddod A (2015) *In vitro* production of date palm *Phoenix dactylifera* L. cv. 'Barhee' plantlets through direct organogenesis. Biological Forum - An International Journal 7: 566-572.

15. Abd El-Baky M A, Abdel-Galeil LM (2016) Effect of different levels of cytokinins on buds proliferation of date palm (*Phoenix dactyliferal*.) cv. Sukary *in vitro*.

16. Rai R (2007) Genetics and plant breeding. Introduction to Plant Biotechnology New Delhi-110012. Pg No: 1-42.

17. Negri V, Tosti N, Standardi A (2000) Slow- growth storage of single node shoots of apple genotypes. Plant Cell Tiss Org Cult 62: 159-162.

18. Engelmann F (2011) Use of biotechnologies for the conservation of plant biodiversity. *In Vitro* Cell Dev Biol Plant 47: 5-16.

19. Sabah AH, Abido AI, Aly MA, Rayan GA (2013) *In vitro* Preservation of Grapevine (*Vitis vinifera* L.) Muscat of Alexandria and Black Monukka Cultivars as Genetic Resource. African J Basi &AppliSci 5: 55-63.

20. Thakur S, Tiwari KL, Jadhav SK (2015) *In vitro* approaches for conservation of *Asparagus racemosus* Willd. *In Vitro* Cell Dev Biol Plant 51: 619-625.

21. El-Dawayati MM, Zayed Z, Baker EI, Gomaa AH (2011) Studies on storage under minimal growth conditions of date palm callus explants. First Scientific Conference for the Development of the Date palm and Dates Sector in the Arab world. King Abdulasis, City for Science and Technology. Kingdom of Saudi Arabia. Pg No: 401-420.

22. El-Dawayati MM, Baker EI, Gomaa AH, Zayed Z (2013) *In vitro* conservation of date palm shoot tip explants under minimal growth condition. Egypt J Agric Res 91: 1043-1062.

23. George EF, Hall MA, Klerk GJ (2008) The components of plant tissue culture media macro and micro-nutrients. Plant Propagation by Tissue Culture Pg No: 65-113.

24. Fortes GR, Pereira JES (2001) Effect of low temperature and growth retardants on *in vitro* conservation of asparagus. Revista Cientifica Rural 6: 181-186

25. Shibli RD, Shatnawi MM, Subaih SS, Ajlouni MM (2006) *In vitro* conservation and cryopreservation of plant genetic resources, a review. World J Agri Sci 2: 372-382.

26. Tyagi RK, Agrawal A, Yusuf A (2006) Conservation of *Zingiber* germplasm through *in vitro* rhizome formation. Sci Hortic108: 210-219.

27. Levitt J (1980) Responses of Plants to Environmental Stresses. Volume 1 Chilling, Freezing and High Temperature Stresses. (2nd edition), Academic Press, New York, USA. Pg No: 497.

28. Graham D, Patterson BD (1982) Responses of plants to low, non-freezing temperatures: proteins, metabolism, and acclimation. Annu Rev Plant Physiol 33: 347-372.

29. Bekheet SA, Taha HS, Saker MM (2001) *In vitro* long-term storage of date palm. Biol Plant 45:121-124.

30. Hassan M (2002) *In vitro* studies on somatic embryogenesis conservation of date palm. Ph.D. Thesis Department of Pomology, Faculty of Agriculture, Cairo University, Egypt.

31. Shibli R, Subaih W, Abdelrahman N (2005) Effect of different carbohydrates on *in vitro* maintenance of date palm embryogenic callus. Adv Hort Sc 19: 172-175.

32. El-Dawayati MM (2008) Using tissue culture technology to storage some plant tissues of date palm. Ph.D. Thesis, Department of Pomology, Faculty of Agriculture, Cairo University, Egypt.

33. El-Ashry AA, Shaltout AD, El-Bahr MK, Abd El-Hamid A, Bekheet SA (2013) *In vitro* preservation of embryogenic callus cultures of two Egyptian dry date palm cultivars at darkness and low temperature conditions. J Hort Sci Ornam Plants 5: 118-126.

34. El-Bahr MK, Abd EL-Hamid AB, Matter MA, Shaltout A, Bekheet SA, et al. (2016) *In vitro* conservation of embryogenic cultures of date palm using osmotic mediated growth agents. J Gen Engi Biotech.

35. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-497.

36. Pottino B (1981) Research on *in vitro* propagation of (*Prunus laurocensis*) cv. Ottowyken. Acta Horticult 300: 177-180.

37. Association of Official Agricultural Chemists (1980) Official Methods of Anal. (13th edition), Association of Official Agricultural Chemists, Washington D.C, USA.

38. Dubois M, Gilles AK, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugar and related substance. Analyte Chem 28: 350-356.

39. Schroder C (1970) Tissue culture of Date Palm Shoots and seedling. Date Grow Inst Rep 47: 25-27.

40. Abebe T, Guenzi AC, Martin B, Cushman JC (2003) Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. Plant Physiol 131: 1748-1755.

41. Kovalchuk I, Lyudvikova Y, Volgina M, Reed BM (2009) Medium, container and genotype all influence *in vitro* cold storage of apple germplasm. Plant Cell Tissue Organ Cult 96: 127-136.

42. Chauhan R, Keshavkant S, Jadhav SK, Quraishi A (2016) *In vitro* slow-growth storage of *Chlorophytum borivilianum* Sant. Et Fernand: a critically endangered herb. *In Vitro* Cell & Dev Biol- Plant 52: 315-321.

43. George EF (1996) Micro propagation in practice. In: Plant propagation by tissue culture. Part 2, (2nd edition), Exegetics Ltd, England.

44. Silva RC, Gomes LZ, Schrewinski-Pereira JE, (2012) Short-term storage *in vitro* and large- temperature storage scale propagation of grapevine genotypes. Pesq agropec bras 47: 344-350.

45. Panis B, Totte NV, Nimmen K, Withers LA, Swennen R (1996) Cryopreservation of banana (Musa spp.) meristem cultures after pre-culture on sucrose. Plant Sci 121: 95-106.

46. Khuri S, Moorby J (1995) Investigations into the role of sucrose in potato cv. Estima microtubers production *in vitro*. Ann Bot 75: 295-303.

47. Yasseen M, Ahmed T, Sablok G, Standardi A, Shadihade AN, et al. (2012) Review: role of carbon sources for *in vitro* plant growth and development. Molec Biol Rep Pg No: 301-4851.

48. Moges A, Karam N, Shibli R (2003) Slow growth *in vitro* preservation of African violet (*Saintpaulia ionantha* Wendl.). Adv Hort Sc 17: 223-230.

49. Baghdadi S, Shibli R, Syouf M, Shatnawai M, Arabiat A, et al. (2011) Medium term preservation of wild Crocus (*Crocus hyemalis* and *Crocus moabiticus*) Embryogenic Callus. Jordan J Agri Sci 7: 482-495.

50. Gianní S, Sottile F (2015) *In vitro* storage of plum germplasm by slow growth. Hort Sci 42: 61-69.

51. Du Y, Li W, Zhang M, He H, Jia G (2012) The establishment of a slow-growth conservation system *in vitro* for two wild lily species. African J Biotechnol 11: 1981-1990.

52. Levitt J (1980) Responses of Plants to Environmental Stresses, Volume 1. Chilling, Freezing and High Temperature Stresses, (2nd edition), Academic Press, New York. Pg No: 497.

53. Graham D, Patterson BD (1982) Responses of plants to low, non-freezing temperatures: proteins, metabolism, and acclimation. Annu Rev Plant Physiol 33: 347-372.

54. Kaur D, Ogra RK, Bhattacharya A, Sood A (2012) Changes in sugar levels during slow growth of *Dendrocalamus hamiltonii* somatic embryos due to liquid paraffin overlay. *In Vitro* Cell & Dev Biol-Plant 48: 120-126.

55. Kushwaha R, Pandey S, Chanda S, Bhattacharya A, Ahuja PS (2007) GA3 induced changes in slow growing endangered Himalayan plant *Podophyllum hexandrum* and hastening of vegetative growth. Plant Growth Regul 51: 207-215.

56. Jo EA, Tewari RK, Hahn EJ, Paek KY (2009) *In vitro* sucrose concentration affects growth and acclimatization of *Alocasia amazonica* plantlets. Plant Cell Tiss Org 96: 307-315.

57. Parida AK, Dagaonkar VS, Phalak MS, Umalkar GV, Aurangabadkar LP, et al. (2007) Alterations in photosynthetic pigments, protein and osmotic components in cotton genotypes subjected to short-term drought stress followed by recovery. Plant Biotechnol Rep 1: 37-48.