Saudi Journal of Biological Sciences 26 (2019) 1896–1902

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Cryopreservation: A tool to conserve date palm in Saudi Arabia

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ARTICLE INFO

Article history:
Received 16 December 2018
Revised 7 February 2019
Accepted 12 February 2019
Available online 13 February 2019

Keywords:
Cryopreservation
Date palm
Embryogenesis
Vitrification
Encapsulation
Dehydration

ABSTRACT

The cryostoring of embryogenic tissue of the date palm (*Phoenix dactylifera* L. cv. Sagai) was examined through dehydrated-encapsulation, vitrification, and vitrification-encapsulation. The most extreme regeneration rate (53.33%) of epitomized, cryostored liquid nitrogen (+LN) treated embryos was observed when pre-embryonic masses were hatched with 0.5 M sucrose for 48 h pursued by 6 h air drying out. The most noteworthy survival rate (80.0%) of epitomized, cryopreserved embryonic cluster came about when calli were hatched with 0.3 or 0.7 M sucrose for 48 h pursued by four hours of lack of hydration, or with 0.5 M sucrose for 48 h without air drying out or with 2 h of air drying out. Following cryopreservation utilizing the embodiment vitrification convention, the most astounding survival (86.7%) as well as the greatest growth (46.7%) was accomplished when the typified vitrified, cryopreserved calli were treated with Vitrification Solution 2 for plants (PVS2) for 60 min at 25 °C. Cryopreservation utilizing the vitrification convention brought about the most extreme recuperation of 53.3%, when vitrified-cryopreserved calli were subjected to PVS2 solution for 30 min at 25 °C. Most extreme (40%) regeneration of vitrified, cryopreserved embryonic calli was seen when these calli were treated with PVS2 solution for 60 min at 25 °C. The outcome got amid this investigation of regrowth after cryopreservation of the cv. Sagai was over the base suitable for a cryo-germplasm bank. Recovery and regrowth were above 30% for all the techniques developed for the cv. Sagai.

1. Introduction

The date palm is viewed as the primary wellspring of nourishment and a noteworthy salary hotspot for the local populace in the Middle East and North Africa. It has expected basic jobs in the economy and society. It is a quick wellspring of sustenance and is likewise used for the treatment of different diseases. Diverse parts of the palm are utilized for different purposes (Chao and Krueger, 2007). The Date palm has intriguing natural and formative properties which have assisted it with multiplying (Hadrami and Hadrami, 2009).

Cryopreservation is a biotechnology procedure which has accepted a critical place in overall plant security programs and the defending of plant innate resources (Bajaj, 1995; Benson, 2002). Cryopreservation at −196 °C is viewed as the favored strategy for long haul protection of plant hereditary sources without changing its qualities (Al-Khayri and Naik, 2017). The cryopreserved materials don't require subculture and hypothetically the hereditary material can stay flawless for an uncertain period (Kaczmarczyk et al., 2008). Cryopreservation can be done by preculture-drying out, epitome lack of hydration, vitrification, exemplification vitrification, and the bead strategy (Engelmann, 2004).

The embodiment lack of hydration system includes epitomizing the explants with a Na-alginate gel (Kaviani, 2011), trailed by drying out which is done either with silica gel or in the wind current of a laminar stream (Paulet et al., 1993). Also, the nutritive covering (the speck) enveloping the explant can propel its regrowth after defrosting.

Fruitful cryopreservation relies upon various factors, like the wellspring of the explant and the affectability of the hereditary plant material to stresses (Reed et al., 2005); crude materials, culture conditions, medications, strategies for cryopreservation, and nature of fluid nitrogen solidifying offices are generally factors which can impact suitability (Reinhoud et al., 2000; Reed et al., 2004). Numerous investigations have shown that some

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2019.02.004
1319-562X/

cryoprotectants prompt expanded solidness of the cell layer through direct communication or through the redistribution of water in and around the cells (Uemura et al., 2009). There are diverse strategies and defensive materials which stay away from the unsafe impacts of solidifying utilized amid cryopreservation. Some molecules such as DMSO, glycerol, and some amino acids and proline easily enter the cell while others don't enter the cell e.g., sugars, expansive particles like PEG. The DMSO is the most appropriate since it enters cells rapidly (Rajasekharan, 2006). Protection of the germplasm of date palm utilizing customary techniques is troublesome due to the high danger of the spread of vermin and pathogens. Cryopreservation is the system most suited for long haul preservation the germplasm (Bekheet, 2011).

Existing exploration concerning the utilization of cryopreservation systems is restricted and just a couple of assortments of date palm have been tried, i.e., cv. Magdool (Subayh et al., 2007), cv. Zaghlool (Bekheet et al., 2007), as also obscure cultivar (Mycock et al., 1995). Past examinations have demonstrated the impact of various parts including PEG, glycerol, DMSO, and sucrose on the preservation (Mycock et al., 1995; Subayh et al., 2007; Tisserat et al., 1985). On account of the extraordinary prerequisites for every genotype (cultivar) of date palm, it is important to work out the specific cryoprotectant arrangement segments for a particular genotype (Ellis et al., 2006).

2. Materials and methods

2.1. Plant material

Grouped parts of *Phoenix dactylifera* L.cv. Sagai were accumulated from the Al-Rajhi Ranch (Al-Qassim) Saudi Arabia. Young branches, 2–3 years old, were expelled from strong mother trees. Sharp tools were utilized to take out shoot tips. The apical tips of shoots were washed with deionized water for 10 min to expel any dirt and immediately set in a chilled oxidation prevention agent (100 mg/L of citric acid, 150 mg/L ascorbic acid) at 4 °C for 24 h in a refrigerator.

Shoot tips were washed with deionized water after treatment with anti-oxidative solution. The trimmed shoot tips were then dipped in 70% ethanol for a few moments. The surfaces of the shoot tips were cleaned in sodium hypochlorite solution (2.0%; 40% v/v Clorox), enhanced with few drops of cleaning detergent for every 100 ml for disinfection (20 min) with sporadic shaking. The tips were then cleaned with sterile water for 15 min, 3–4 times with delicate shaking. Following Bekheet et al. (2007) apical meristem was cleaned and refined in adjusted MS medium (Murashige and Skoog, 1962). Calli appeared after 1.5–3.5 month of incubation. Then calli moved to another, altered MS medium enhanced with 0.1 mg/L 2,4-D for enlistment of embryogenic callus. The embryonic calli were developed on the MS medium free of hormones to get adequate embryonic callus (three months old culture). The callus tissues were grown at 27 ± 2 °C, for a light regime of 16 h light/8h dark cycle by cool white tube lights at 50–60 μmol/m^2 photosynthetic photon flux density (PPFD).

2.2. Cryopreservation

2.2.1. Encapsulation-dehydration

Embryogenic callus precultured for two days on MS medium containing 0.5 M sucrose. The embryonic calli were cut into little pieces (<3 mm) using sharp blade and forceps. The calli of embryogenic capacity were withdrawn together with some alginate medium with the assistance of 10 ml sterile wide mouth pipette and precisely dropped into the MS medium upgraded with 100 mM calcium chloride and 0.1 M sucrose. The embryogenic callus bunches with alginate were kept in the medium for 15 min to ensure desirable polymerization to outline beads of around 4 mm. The alginate beads containing embryogenic callus, were traded to 200 ml beaker containing 50 ml liquid MS media free from PGRs supplied with 0.1, 0.3, 0.5, 0.75 or 1.0 M sucrose. The flasks were rotated on a shaker at 100 rpm/min for two days for drying out.

Along these lines, the absence of hydration carafes was released using a perfect pipette and the dabs (assembled synseeds) were dried in a laminar stream air current for 0, 2, 4, 6, or 8 h, on sterile paper in a Petri plate at 25 °C. Half of the synseeds (5 beads) from each treatment were subjected MS medium with 0.1 mg/l 2,4-D for rehydration and progression (as a control). The other half, were set in 2 ml, clean, named cryo-vials (each containing five beads) and clearly brought into liquid nitrogen (LN) 48 h.

After cryopreservation in LN, the cryogenic vials containing alginate beads were defrosted on a water bath at 38 °C for 2–4 min. The cryopreserved synseeds were exposed to MS medium containing 0.1 M sucrose for three days. After this the dried non-cryopreserved (−LN) calli and exemplified, dried cryopreserved (+LN) embryogenic calli of date palm were traded to regular headway conditions. After one and a half months the calli were examined visually (colour and size of the callus); darker beads seized to grow. In addition, the TTC test was also done for viability.

The moisture content (MC) and absence of hydration, the beads without embryogenic callus were managed and investigated as in advance depicted. The beads to be dried were evaluated (FWt0) at a beginning time point and all through the drying out time (FWtx). Finally, the beads were dried in an oven at 70 °C for 48 h and reweighed (DW). The moistness of the bead was figured after a given drying period (tx).

MCtx (% moistness content) = ((FWtx − DW)/FWt0) × 100 (Gonzalez-Arnao and Engelmann 2006).

2.2.2. Vitrification

The precultured (detailed earlier) embryogenic callus groups were moved to cryogenic vials containing a stacking plan (LS, 2 M glycerol, 0.4 M sucrose in MS medium) for 25 min (Panis et al. 2005). The calli were dried by ice cooled plant vitrification solution (PVS2) (Sakai et al. 1990).

The embryogenic calli were treated in PVS2 solution at 25 °C for 0, 15, 30, 60, or 120 min. Half of the embryogenic callus bundles in the cryogenic vials with the vitrification course of action were submerged into LN for a minimum 48 h. After cryopreservation in LN, the embryogenic calli were defrosted on water bath at 38 °C for 2– 3 min. The PVS2 course of action was displaced (with MS liquid medium comprising 1.2 M sucrose) at the room temperature for 15 min. Then the embryogenic callus groups, with and without LN treatment, were traded onto the recovery medium (gelled MS medium containing 0.5 M sucrose) and incubated for three days.

As such, the embryogenic callus bunches were subjected to the MS medium fortified with 30 g/L sucrose and 0.1 mg/L 2,4-D, under standard conditions. Following 1.5 months, survival and regeneration of the embryonic calli, of cryoprotected and non-cryoprotected were recorded.

2.2.3. Encapsulation-vitrification

As described above precultured embryonic calli were cut into little pieces using scalpel blade and forceps (<3 mm) and submerged into the MS liquid medium lacking PGRs as well as calcium chloride. The MS liquid medium was supplied with 0.4 M sucrose, 2.0 M glycerol and 3.0% (w/v) sodium alginate. The embryonic callus bunches were restricted together with some alginate using a 10 ml sterile pipette and carefully trickled large drops into the liquid MS medium having 0.4 M sucrose and 90 mM calcium chloride (Lakshmana and Singh 1990); then kept for 15–20 min for

polymerization to outline beads around 4 mm dia. Ensuing that each encapsulating has one embryogenic callus, was then treated with the vitrification procedure delineated previously.

2.2.4. Experiment design and statistics

All analyses were done by a completely randomized structure. All medications were connected three times with five embryogenic callus bunches for each duplicate. The information are communicated as rates; the change of the rate information was a bit much. Measurable information was investigated utilizing SPSS (adaptation 11, SPSS Inc. Chicago, USA) programming bundle. Means were looked at by the investigation of fluctuation (ANOVA), as per Duncan's test at a 0.01% significance level.

3. Results and discussion

3.1. Encapsulation-dehydration

The effect of absence of hydration period and sucrose obsessions in the midst of preculture on the survival and regeneration rates of exemplified, cryopreserved (+LN), and non-cryopreserved (−LN) embryonic calli of date palm cv. Sagai refined in vitro is shown in Table 1 and sketched out in Fig. 1. There were basic differentiations between different centralizations of sucrose in the midst of preculture and parchedness period in their effect on the rates of survival and growth of exemplified cryopreserved (+LN) and non-cryopreserved (−LN) embryonic calli.

The data (Table 1) showed that an aggregate loss of regrowth for embodied cryopreserved (+LN) embryogenic calli of cv. Sagai and the calli were grown with 0.1 or 1.0 M sucrose for two days without air drying or with 1.0 M sucrose sought after 8 h of air drying out. The aggregate loss of regrowth by virtue of exemplified cryopreserved (+LN) embryogenic calli with 8 h of air absence of hydration may be a result of over the best drying out. In any cryogenic technique, finding the perfect time of air absence of hydration requires setting up the perfect water content in cells to such a degree, to the point that cryopreservation can stumble over snappy cooling in LN without encountering lethal intracellular hardening. The nonappearance of regrowth by encapsulated, cryopreserved (+LN) embryogenic calli without air and hydration, might be an immediate after effect of the course of action of ice valuable stones, both extra and intra cell, in the light of a high clamminess content (Plessis et al., 1993; Moges et al., 2003). The water substance of globules agonized with 0.1 or 1.0 M sucrose for two days without air parchedness were 93.83% or 69.38%, independently. These results are as per (Subaih et al., 2010). They demonstrated nonattendance of regrowth for epitomized, cryopreserved (+LN) calli of date palm cv. Medjool happened when calli were exposed to 0.1–0.3 M sucrose for two days without air drying, where the water substance of embodied cryopreserved calli subjected to 0.1 and 0.3 M sucrose (92.5% and 87.2%, separately).

The most critical survival rate (80%) of encapsulated cryopreserved embryogenic calli of cv. Sagai was gotten as the calli were brought forth on to 0.3 or 0.7 M sucrose for two days sought after by 4 h of absence of hydration, or with 0.5 M of sucrose for two days without air drying out or 2 h of drying out (Table 1). These results resemble those of Subaih et al. (2007). They found that the most survival rate (80%) of cryopreserved (+LN) calli of date palm cv. Medjool was practiced when calli were precultured with 0.3 M sucrose for two days after 2 h of dehydration where the harmful substance of globules was diminished to 55.4%. Moges et al. (2003) found 100% survival of exemplified, cryostored of African violet shoot tips (*Saintpaulia ionantha* Wendl.) was cultivated when precultured with 0.3 M sucrose at two days duration for 2 h of drying. It is crucial that in this examination a segment of the typified, cryopreserved embryogenic calli endure and regrew following a half year of subculture on the regrowth medium. This may be a direct result of the finish of cell assimilation which is basically zero at −196 °C (Benson, 2008). After recovery of the encapsulated, cryopreserved embryogenic calli from LN, cell

Table 1
Percentage of the survival and regrowth for encapsulated cryopreserved (+LN) and non-cryopreserved (-LN) embryogenic calli of date palm cv. Sagai using encapsulation-dehydration technique.

Sucrose Conc. (M)	Dehydration Period (h)	Cryopreserved (+LN)		Non-cryopreserved (-LN)	
		Regrowth (%)	Survival (%)	Regrowth (%)	Survival (%)
0.1	0	0.00 a	66.67 de	86.67 h	100 g
	2	20 cd	60 cd	80 g	86.67f
	4	33.33 ef	73.33 ef	73.33 g	86.67f
	6	40 fg	60 cd	60f	80 ef
	8	6.67 ab	53.33 bc	40 d	66.67 cd
0.3	0	6.67 ab	66.67 de	73.33 g	86.67f
	2	33.33 ef	73.33 ef	73.33 g	80 ef
	4	40 fg	80f	60f	73.33 de
	6	46.67 gh	60 cd	53.33 ef	73.33 de
	8	6.67 ab	53.33 bc	26.67c	46.67 a
0.5	0	20 cd	80f	73.33 g	86.67f
	2	40 fg	80f	60 d	80 ef
	4	33.33 ef	73.33 ef	53.33 ef	73.33 de
	6	53.33 h	66.67 de	46.67 de	66.67 cd
	8	13.33 bc	33.33 a	26.67c	60 bc
0.75	0	13.33 bc	66.67 de	60f	73.33 de
	2	33.33 ef	60 cd	53.33 ef	73.33 de
	4	40 fg	80f	46.67 de	66.67 cd
	6	46.67 gh	73.33 ef	46.67 de	66.67 cd
	8	13.33 bc	60 cd	6.67b	46.67 a
1	0	0.00 a	53.33 bc	46.67 de	66.67 cd
	2	20 cd	73.33 ef	46.67 de	66.67 cd
	4	33.33 ef	60 cd	33.33c	60 bc
	6	26.67 de	46.67b	26.67c	53.33 ab
	8	0.00 a	26.67 a	0.00 a	46.67 a

*Means within column having different letters are significantly different according to (Duncan's test at $P \leq 0.01$).

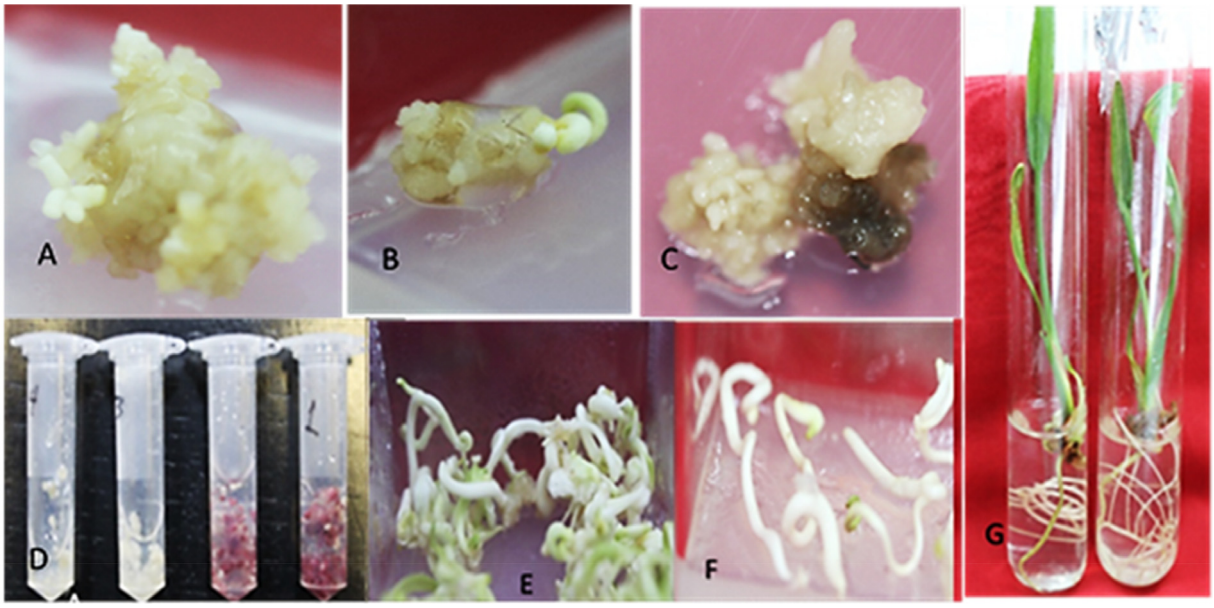

Fig. 1. Regeneration of cryopreserved (+LN) embryogenic calli of cv. Sagai after six weeks from culture on recovery MS medium. (a) Using technique Encapsulation-dehydration. (b) Using technique Encapsulation-vitrification. (c) Using technique vitrification. (d) Test viability of embryogenie callus after cryopreservation using triphenyl tetrazolium chloride (TTC). (e) and (f) Production huge somatic embryos from embryogenic callus after cryopreservation. (g) Rooted plantlet obtained from a somatic embryo regenerated from cryopreserved embryogenic callus.

absorption step by step reactivated and required somewhere in the range of a chance to proceed run of the mill assimilation (Heringer et al., 2013).

The best regrowth (53.33%) of exemplified cryopreserved (+LN) embryogenic calli of cv. Sagai in this work was noted when agonized with 0.3, 0.5 or 0.75 M of sucrose for two days sought after by 6 h air absence of hydration (Table 1). Contrary to these disclosures, Subaih et al. (2007) found the most hoisted regrowth (33.3–40%) of epitomized, cryopreserved calli cv. Medjool with 0.1 M sucrose sought after by 2 or 4 h of drying out, with 0.3 M sucrose sought after by 2 h of absence of hydration, or with 0.5 M sucrose with 4 h of drying out. A high regrowth rate of encapsulated, cryopreserved bounce (*Humulus lupulus* L.) tips of shoot was cultivated after two days of preculture in sucrose and 4 h of dehydration (Martinez and Revilla, 1998).

In the present examination, survival and regrowth rates of synseed, cryopreserved calli kept an eye on augmentation with growing sucrose up to 0.7 M and with range of absence of hydration up to 4 h for survival or up to 6 h drying out for regrowth (Table 1). These data may exhibit that the union of sucrose in the preculture medium would incite building up of solutes inside the cell assure the maintenance of plasma membrane and inner layers during drying out, up to 6 h, and setting in LN (Plessis et al., 1993; Moges et al., 2003).

A high potential of sucrose in plant cells is favorable in working up a vitrified status during cementing, so keeping the damage caused by drying (Wang and Deng, 2004). Nevertheless, the water substance of touches after treatment with 0.1 or 1 M sucrose sought after by evaporating out to 4 h was some place in the scope of 59.3 and 42.5% while, the water substance of spots after treatment with 0.1 or 1 M of sucrose sought after by absence of hydration up to 6 h hours was some place in the scope of 39.5 and 26.9%. Despite these revelations, Subaih et al. (2007) uncovered that regrowth and survival of encapsulated, cryopreserved calli of cv. Medjool watched out for augmentation with extending gathering of sucrose up to 0.3 M and the season of absence of hydration up to 4 h.

Regrowth and survival rate decreased at all combinations of sucrose when exemplified, cryopreserved calli of cv. Sagai were got dried out with or without air parchedness up to 8 h. This may be a direct result of over the best absence of hydration which may cause cell destruction due to dynamic plasmolysis or osmotic daze (Gonzalez-Arnao et al., 2010). Where the water substance of specks after the treatment with 0.1 or 1 M of sucrose, trailed by evaporating out to 8 h was some place in the scope of 24.1 and 15.0%. On the other hand, the water substance of specks after 0.1 or 1 M of sucrose treatment without absence of hydration was some place in the scope of 93.8 and 69.4%, and this may cause the improvement of intra and extra-cellular crystals of in the light of high moistness (Moges et al., 2003). Al-Ababneh (2001) found that no survival of exemplified, cryoprotected shoot tips was in absence of hydration within synseed gel the moisture level of 75–85%.

The perusal of data (Table 1) revealed that the endurance and regeneration rates for the encapsulated, non-cryopreserved (- LN) calli were diminished with growing drying out period, especially at 1.0 and 0.75 M sucrose treatment. This might be credited to osmotic shock at higher sucrose potential and extravagant drying out that may cause cell end in view of dynamic plasmolysis or osmotic stress (Wilkinson et al., 2003; Gonzalez-Arnao et al., 2010). These results are maintained by the past work of Subaih et al. (2010) who noted that extended time of absence of hydration and assembly of sucrose caused a decrease in survival and regeneration rates of non-cryopreserved and embodied calli of the date palm cv. Medjool. They found that an aggregate loss of regrowth limit happened while using 1.0 M sucrose for two days.

The revelations in Table 1 demonstrate that the gathering of sucrose and drying out period were on another way concern to the survival and regeneration rates of gel covered, non-cryopreserved calli. This may be deciphered as the water substance of cells being satisfactory for them to make due without damage in the midst of treatment. The water content in synseeds treated with 0.1, 0.3, or 0.5 M sucrose for 48 h after non-air drying out or with 2 and 4 h air drying was between (93.8–49.0%).

The most vital survival rates (86.7 to 100%) of embodied non-cryopreserved calli were noted when they were dried with 0.1, 0.3 and 0.5 M sucrose for two days without air drying out, or with 2 and 4 h air absence of hydration because of 0.1 M sucrose (Table 1). However, the most pronounced regrowth rates (73.3 to 86.7%) of encapsulated, non-cryopreserved calli were gotten when calli were dried out with 0.1, 0.3, or 0.5 M sucrose without hydration, or with two and four hours of air drying out for 0.1 and 0.3 M sucrose independently.

Our results are as per Subaih et al. (2010) who referred to that the most extraordinary survival (93.3–100%) of epitomized, non-cryopreserved calli cultivated brought forth with 0.1 or 0.3 M sucrose for two days with or without 2 h of air drying. They observed that the best regrowth (73.3–80%) of calli was attained when calli were treated with 0.1 or 0.3 M sucrose, with or without 2 h of drying.

3.2. Vitrification

The effect of absence of hydration with concentrated PVS2 on the rates of survival and regeneration of vitrified, non-cryoprotected (−LN) and cryoprotected (+LN) embryonic calli of date palm cv. Sagai is shown in Table 2 and appeared in Fig. 1. It is evident that there were enormous effects of different lengths of drying out with PVS2 on rates of survival and regeneration.

After cryopreservation, survival, and regeneration rates of vitrified cryopreserved (+LN) embryonic calli extended with growing bring forth period with accumulated PVS2 at 25 °C from 0 to 30 min post survival (53.33%) and 60 min for regrowth (40.0%). Survival and regeneration rates by then decreased with growing treatment time with PVS2 course of action up to 120 min (Table 2). Right when callus was not treated with PVS2 course of action, after cryopreservation, the survival and regeneration rates of vitrified, cryopreserved (+LN) embryonic calli were 40% and 0%, independently (Table 2).

The illumination for these disclosures may be that the perfect term of treatment with collected PVS2 course of action at 60 min gives sufficient absence of hydration for calli to keep the improvement of ice valuable stones inside cells when cryopreserved in LN. When calli were brought forth in PVS2 they responded in due order regarding periods under 60 min, drying out was lacking to turn away water valuable stone game plan, achieving ice pearl gathering inside the telephones and provoking lessens in the rates of regrowth in the wake of refined on recovery medium (Mycock et al., 1995). In any case, the clarification behind extended survival when calli were treated with PVS2 for 30 min may be required to non-savage mischief (cryo-harm) in the midst of the cryopreservation which hampered the regrowth. On the other hand, when calli were dried in collected PVS2 at 25 °C for more than 60 min causing a reducing in the rates of both survival and regeneration, extreme absence of hydration and furthermore risky effects of dimethyl sulfoxide (DMSO) may have been the reason.

Table 2
Percentage of the survival and regrowth for cryopreserved (+LN) and non-cryopreserved (−LN) embryogenic calli of Date palm cv. Sagai using vitrification technique.

PVS2 (min)	+LN		−LN	
	Survival (%)	Regrowth (%)	Survival (%)	Regrowth (%)
0	40 bc	0 a	100 e	100 e
15	46.67 bc	13.33 bc	93.33 de	73.33 d
30	53.33c	33.33 d	86.67 cd	66.67 cd
60	46.67 bc	40 d	80 bc	60 bc
90	33.33b	20c	73.33 ab	53.33b
120	13.33 a	6.67 ab	66.67 a	26.67 a

*Means within column having different letters are significantly different according to (Duncan's test at $P \leq 0.01$).

Our results are strangely with Moges et al. (2003) who uncovered the best regrowth (90%) of African violet shoot tips were dried with a four-phase methodology of extending gradual increase of concentration of PVS2, all shoot tips survived when cultivated after cryopreservation, paying little notice to the mixing of PVS2. Diverse makers (Suranthran et al., 2012) communicated that in the cryostorage of oil palm (*Elaeis guineensis*, Arecaceae) presentation of poly-embryoids to PVS2 for more than 10 min caused their short-lived. Also, (Heringer et al., 2013) showed that peach palm physical creating lives were exceedingly tricky to DMSO. Thusly, they used PVS3 vitrification course of action (50:50, sucrose: glycerol) and after cryopreservation no regrowth was observed, when the agonizing time was reduced 60 min or no incubating. Regardless, they reported that the perfect agonizing time in PVS3 was 240 min, which gave a regrowth rate of 25.9% −37.0% following 12 weeks. Thus, the perfect time of bring forth in vitrification course of action is dependent on species, and, on the weight and size of explants and the kind of vitrification game plan (Niino et al., 1992).

On the other hand, our disclosures are close with those of Fki et al. (2013). They found that perfect PVS2 treatment was 30 min when they used standard vitrification traditions. After cryopreservation, the most raised recuperation rate was 26.7%, for date palm cv. Khenizi caulogenic meristems.

This is clear from the data displayed in Table 2 that using the standard vitrification tradition, the perfect time period for vitrification with PVS 2 was 30 min for survival rate and 60 min for regrowth rate after cryopreservation. The rates of survival and regeneration of the vitrified, non-cryotreated (−LN) embryonic calli are presented in Table 2 and show that survival and regrowth limit lessened with extending time of absence of hydration with PVS2 from 0 min to 120 min. The most prominent survival and regeneration level of vitrified, non-cryoprotected (−LN) embryogenic calli (100%) was obtained, when the calli were not subjected to PVS2. While, the minimum survival (66.7%) and the base regrowth (26.7%) were seen when the vitrified non-cryopreserved (−LN) embryogenic calli were treated with PVS2 for 120 min.

The reason behind the decreased regrowth and survival rates of vitrified, non-cryopreserved (−LN) embryogenic calli with growing bring forth period with amassed PVS2 at 25 °C from 0 to 120 min may be an aftereffect of the blocking effects of DMSO and super osmotic drying out. The control treatment (with no PVS2) demonstrated the best regeneration and survival of 100% for every circumstance and this might be because of the sucrose preculture and stacking plan attempted was nontoxic. Subaih et al. (2010) found an imperative effect on survival and regeneration of non-cryoprotected calli of cv. Medjool with different centralizations of PVS2. They similarly communicated that the best survival and regrowth rates of 66.7% and 33.3%, independently, were gotten for non-cryopreserved calli treated with amassed PVS2 at 25 °C for 20 min. The differentiation between their results and our very own strength be a result of the qualification in the combinations inspected, presentation period for PVS2 and the gathering of sucrose in the preculture. They used 0.3 M sucrose and we used MS medium comprising 0.5 M sucrose. Contrary to the disclosures in this work, Moges et al. (2003) nitty gritty no immense assortments to the extent regrowth and survival of non-cryopreserved shoot tips of African violet where whole survival and regeneration of shoots were found for non-cryopreserved shoot tips, free of PVS2 solution immersion.

3.3. Encapsulation vitrification

The effect of absence of hydration term with PVS2 on the rates of survival and regeneration of dried vitrified, cryoprotected (+LN)

and non-cryoprotected (−LN) embryonic calli of date palm cv. Sagai is shown in Table 3 and in Fig. 1. There were important assortments in survival and regrowth rates.

The data (Table 3) exhibit that after cryopreservation survival and regrowth limit extended with growing time of absence of hydration with concentrated PVS2 from 0 to 60 min for exemplified vitrified cryopreserved calli and subsequently reduced with extending agonizing period from 60 to 120 min Table 3. The most surprising survival rate (86.7%) and most prominent regrowth (46.7%) were obtained when the exemplified vitrified, cryopreserved calli were treated with concentrated PVS2 for 60 min. It is fundamental to observe that after cryopreservation, when PVS2 was not used, the survival and regrowth rates of embryogenic calli were 6.67% and 0%, exclusively. This demonstrates the perfect term of treatment with concentrated PVS2 was 60 min, giving satisfactory absence of hydration to keep the advancement of ice. When exemplified calli were brought forth in concentrated PVS2 for different periods under 60 min, the absence of hydration periods was not sufficient and during cryopreservation ice pearls may have surrounded inside cells causing lessens in survival and regrowth after culture of encapsulated, cryopreserved calli on recovery medium. Agonizing in amassed PVS2 at 25 °C for more than 60 min realized a reducing in survival and regrowth after culture of exemplified calli on recovery medium and this may be a direct result of outrageous drying out and also risky effects of DMSO.

In advance, the effect of sucrose preculturing on the cryopreservation of date palm (Fki et al., 2013) was seemed to cause extended proline content in date palm tissues. Pociecha et al. (2008) declared upgraded security from both genuine osmotic weight and low temperature was through the specific social occasion of this amino destructive. The effect of sucrose on banana meristem preculture on protein absorption was analyzed by means of Carpentier et al. (2010) by two-dimensional gel electrophoresis. These makers found that sucrose preculture could change the characteristics which are required for cementing obstruction.

The results in our examination are as per Fki et al. (2013) who found that, when the embodiment vitrification tradition was used, the perfect term of treatment in PVS2 extended to 60 min when recuperation after cryopreservation was 60% for date palm cv. Khenizi meristems leading to callus formation.

Instead of our disclosures, Subaih et al. (2010) uncovered that, post cryopreservation, the most extraordinary survival (60%) of calli was gotten when encapsulated calli of date palm cv. Medjool was got dried out with accumulated PVS2 at 25 °C for some place in the scope of 5 and 10 min, and there was no assortment in the regrowth rate (26–20%) in light of parchedness period. In addition, Al-Ababneh et al. (2002) found the most extraordinary revival of cryopreserved shoot tips subsequent to drying out with concentrated PVS2, at 0 °C for 2–3 h. Moges et al. (2003) observed most extraordinary revival (80–85%) and regeneration (70–80%) of cryopreserved African violet were gotten when typified shoot tips were got dried out with accumulated PVS2 at 25 °C for some place in the scope of 5 and 30 min.

It is obvious from the data shown in Table 3 that, using the standard encapsulation vitrification, the perfect time for vitrification with concentrated PVS2 was 60 min for the rates of survival and regrowth after cryopreservation of vitrified calli of date palm cv. Sagai. The survival and regrowth rates of dried vitrified, non-cryopreserved (−LN) embryogenic calli were lessened with growing drying period at 25 °C from 0 to 120 min (Table 3).

The most visible survival (100%) and the best regrowth (93.3%) of synseed vitrified non-cryopreserved calli were gotten when not treated with PVS2. While, the most lessened survival (66.7%) and the base regrowth (40.0%) were seen when the encapsulated vitrified non-cryopreserved calli were treated with PVS2 for 120 min. This may be a result of extending osmotic shock or harmful effects of DMSO or may owe extraordinary absence of hydration of the cells. These results are in simultaneousness with Salma et al. (2014) when the globule cryo-plate system was associated with non-sucrose precultured and non-cryopreserved (−LN) PEMs, in cv. Sokary. The most vital recovery obtained was 71.1% for no absence of hydration, by then reduced to 0% after 300 min drying out. They in like manner, itemized that for the arrangement Sultany, regrowth was 73.3% for no absence of hydration and decreased to 26.8% after 300 min. In any case, then again, Subaih et al., (2010) found that absence of hydration period had no effect on survival. Additionally, regrowth was 60.0–73.3% regrowth of the encapsulated vitrified, non-cryoprotected calli of cv. Medjool, was noted when calli were dried out with PVS2 at 25 °C for ½ h to 1 h. A couple of differentiations were noted between our results and those gotten by Salma et al. (2014) with PEMs of the cvs. Sultany and Sokary using the cryo-plate strategy; this may be a direct result of using different systems and what's more the particular groupings considered.

Table 3
Percentage of the survival and regrowth for encapsulated cryopreserved (+LN) and non-cryopreserved (−LN) embryogenic calli of date palm cv. Sagai using encapsulation-vitrification technique.

PVS2 (min)	+LN		−LN	
	Survival (%)	Regrowth (%)	Survival (%)	Regrowth (%)
0	13.33 a	0 a	100 e	93.33 e
15	53.33b	20 bc	93.33 de	73.33 d
30	66.67c	40 d	86.67 cd	66.67 cd
60	86.67 d	46.67 d	80 bc	60 bc
90	73.33c	26.67c	73.33 ab	53.33b
120	53.33b	13.33b	66.67 a	40 a

*Means within column having different letters are significantly different according to (Duncan's test at $P \leq 0.01$).

4. Conclusions

The data in (Tables 1, 2 and 3), showed that after cryopreservation, the regrowth and survival rates of the representation parchedness and epitome vitrification techniques were more than those for the vitrification strategy (without alginate). These results avow those got previously by Fki et al. (2013) who defined that gell-alginate was not risky to date palm meristems and beneficial to hold them against cryo-damage.

Acknowledgement

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RGP-014.

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