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BRIEF COMMUNICATION

In vitro long-term storage of date palm

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Abstract

A reliable method for the long-term conservation of date palm tissue cultures is described. *In vitro* shoot bud and callus culture were successfully stored for 12 months at 5 °C in the dark. At this conditions high percent of cultures remained viable without serious signs of senescence. However, the growth rate decreased as storage period increased. The role of sorbitol as osmotic agent in storage was examined. Health shoot bud cultures were obtained after 6 months of storage on medium containing 40 g dm^{-3} sorbitol. This period extended for 9 months in case of callus cultures.

Additional key words: osmotic stress, *Phoenix dactylifera*.

Seeds offer a convenient and reliable means for long-term storage. However, many plants produce recalcitrant seeds that are viable for only short periods. Also, some species are vegetatively propagated. Plant *in vitro* technology offers a long-term conservation of germplasm (Grout 1990). Protoplasts, single cells, and organized tissues (meristems, somatic embryos) can be stored (Bajaj 1983). There are two methods of long-term storing *in vitro* plant cultures: slowing down or suspending growth. Slow growth is achieved by modifying the culture medium or reducing temperature (Withers 1991). Suspension of the growth of *in vitro* cultures can be achieved by cryopreservation, which could results in indefinite storage. The purpose of this study is to devise a simple and reliable method for *in vitro* storage of date palm cultures through slowing growth by reducing temperature and by osmotic stress.

Offshoots of female date palm (*Phoenix dactylifera* L. cv. Zaghlool) were used. Leaves were gradually removed and shoot apices 5 cm in length were taken and kept in an anti-oxidant solution (100 mg dm^{-3} ascorbic acid and 150 mg dm^{-3} citric acid). Explants were then sterilized using 70 % ethanol for 1 min and 2.6 % sodium hypochlorite for 20 min and rinsed three times with sterile distilled water. Shoot tips trimmed to about 1 cm were excised with part of submeristematic tissues and aseptically cultured on Murashige and Skoog (MS) medium supplemented with 2 mg dm^{-3} dimethylaminopurine (2ip) + 1 mg dm^{-3} naphthaleneacetic acid (NAA). Cultures were then incubated in darkness and recultured on same fresh medium every six weeks. After five months, white and nodular cultures were observed. For shoot buds proliferation, nodular cultures were subcultured on MS-hormone-free medium. For induction of callus, cultures were transferred into MS medium supplemented with 10 mg dm^{-3} 2,4-dichlorophenoxyacetic acid (2,4-D) + 3 mg dm^{-3} 2ip.

To investigate the effect of low temperature on storage of date palm tissue cultures, the proliferated shoot buds (2 cm length) were transferred to jars (80 × 40 mm) containing proliferation medium (MS-hormone free) and then incubated at 5 °C in the dark. Survival, healthy shoots and vitrified shoots percentages, number of proliferated shoots and shoots height were recorded from ten replicates after 3, 6, 9 and 12 months of storage. In further experiment, equal pieces (250 mg) of callus were transferred into callus induction medium (MS + 10 mg dm^{-3} 2,4-D + 3 mg dm^{-3} 2ip) and then incubated at 5 °C in the dark. Fresh mass, dry mass, dry mass/fresh mass ratio, and percentage of browning were recorded after 3, 6, 9 and 12 months of storage.

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Abbreviations: MS - Murashige and Skoog; 2,4-D - 2,4-dichlorophenoxyacetic acid; 2ip - dimethylaminopurine; NAA - naphthaleneacetic acid.

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To evaluate the role of sorbitol as osmotic agent in storage of date palm tissue cultures, shoot buds (2 cm length) were cultured on MS-hormone-free medium supplemented with 40 g dm^{-3} sorbitol. Survival, healthy, vitrification percentages, number of proliferated shoots and height of shoots were recorded after 3, 6, 9 and 12 months of incubation at normal growth conditions. For the same purpose, calli (250 mg) were cultured on (MS + 10 mg dm^{-3} 2,4-D + 3 mg dm^{-3} 2ip) + 40 g dm^{-3} sorbitol and then the cultures were incubated at normal growth conditions. Growth dynamics of callus as presented before and percentage of browning were recorded after 3, 6, 9 and 12 months.

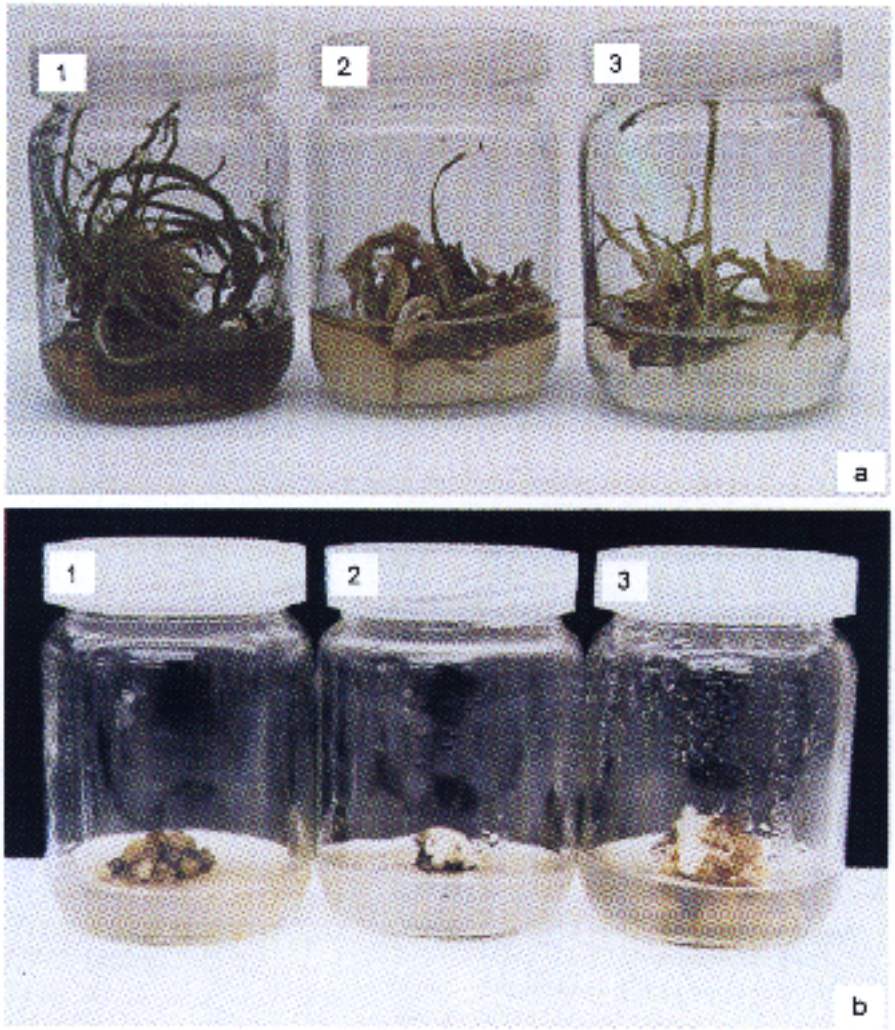

Fig. 1. Shoot cultures (*a*) and callus cultures (*b*) of date palm after three months of storage on normal growth conditions (*1*), medium contained 40 g dm^{-3} sorbitol (*2*) and at 5 °C in the dark (*3*).

Table 1. Survival and proliferation of date palm shoot bud cultures after storage at low temperature of 5 °C or at osmotic stress induced by 40 g dm^{-3} sorbitol. Means ± SE, $n = 10$.

Storage	Survival [%]		Healthy shoots [%]		Vitrified shoots [%]		Prolif. shoot number		Height of shoots [cm]	
[months]	5 °C	sorbitol	5 °C	sorbitol	5 °C	sorbitol	5 °C	sorbitol	5 °C	sorbitol
3	100	100	100	90	-	10	4.80±0.20	4.50±0.10	2.60±0.05	2.50±0.05
6	100	70	90	60	10	10	5.75±0.23	4.90±0.10	3.50±0.15	3.00±0.10
9	80	30	60	-	20	20	5.00±0.20	2.00±0.20	4.60±0.30	3.50±0.30
12	70	-	50	-	20	-	3.00±0.18	-	6.90±0.20	

Table 2. Date palm callus cultures after storage at low temperature of 5 °C or at osmotic stress induced by 40 g dm^{-3} sorbitol. Means ± SE, $n = 10$. Initial fresh mass 250 mg.

Storage [months]	Fresh mass [g]		Dry mass [mg]		Dry mass/fresh mass		Browning [%]	
	5 °C	sorbitol	5 °C	sorbitol	5 °C	sorbitol	5 °C	sorbitol
3	1.65±0.25	1.00±0.15	165.00±10.0	111.11±15.00	0.10	0.11	-	10
6	1.95±0.30	0.75±0.20	177.27±20.00	93.75±10.00	0.09	0.12	10	20
9	2.10±0.20	0.30±0.10	175.00±12.00	37.50±9.00	0.08	0.12	20	30
12	2.00±0.12	-	166.00±13.00	-	0.08	-	20	-

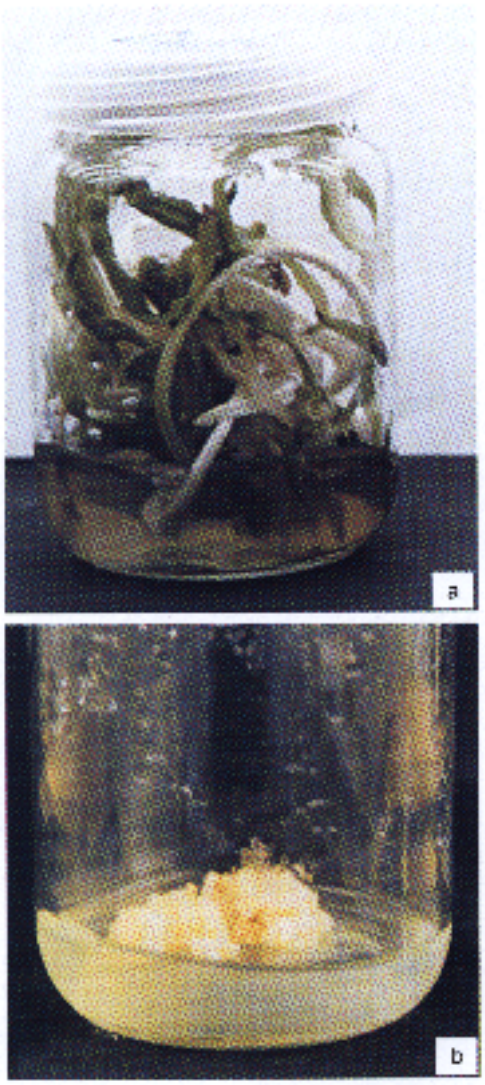

Fig. 2. Date palm shoot cultures (*a*) and callus cultures (*b*) stored for 12 months at 5 °C in the dark.

The media used contained 30 g dm^{-3} sucrose and 7 g dm^{-3} agar and were adjusted to pH 5.8 before autoclaving. The normal incubation conditions were: temperature of 25 ± 2 °C, 16-h photoperiod, irradiance of 45 μmol m^{-2} s^{-1} (*Philips* white fluorescent tubes). Experiments were designed in completely randomized design and obtained data were statistically analyzed according to the method described by Snedecor and Cochran (1967).

Complete survival and 100 % of healthy shoot cultures with no vitrification were registered when shoot bud cultures were stored three months at 5 °C in the dark (Table 1, Fig. 1*A*). 70 % of shoot cultures survived for 12 months, but some cultures showed vitrification (Table 1). At these conditions, the highest rate of shoot proliferation was noticed after 6 months. The maximum height of shoots were observed after 12 months of storage since the cultures were sufficient to fill the jars (Fig. 2*A*).

Callus growth determined as fresh mass gradually increased till 9 month of storage at 5 °C in the dark and then declined (Table 2). However, dry mass and dry mass/fresh mass ratio decreased after 6 months of low-temperature storage. Callus cultures remained fully viable with slight browning after 12 months of storage at 5 °C in the dark (Fig. 2*B*). High percentage of date palm tissue cultures incubated for different periods at 5 °C in the dark were healthy and viable without serious signs of senescence. These results are in accordance with those obtained by Wannas *et al.* (1986) and Wannas (1992). They reported that 85 % of pear and apple shoot cultures remained alive after 18 months of *in vitro* storage at 8 and 4 °C, respectively, in the dark. In this respect Fletcher (1994) and Bekheet (2000) mentioned that, tissue cultures of *Asparagus officinalis* remained viable after 16 and 18 months of storage at 6 and 5 °C, respectively.

Modification of storage medium by incorporating 40 g dm^{-3} sorbitol as osmotic agent was also investigated. 100 % of survival and 90 % of healthy shoots with only 10 % of vitrification and reduced growth rate were observed after three months of storage on hormone-free medium containing 40 g dm^{-3} sorbitol at normal growth conditions. However, survival percent sharply decreased after 6 months and the cultures dried and died after 12 months (Table 1, Fig. 1*A*).

Callus cultures took similar trend (Table 2). However dry mass/fresh mass ratio increased till the sixth month and then decreased. The percentage of browning was higher in callus cultures stored on medium contained 40 g dm^{-3} sorbitol compared with same type of cultures stored at 5 °C in the dark (Table 2, Fig. 1*B*). All callus cultures of date palm dried and died at 12 months. The results are in line with reported by Fletcher (1994). In this

respect, Tisserat *et al.* (1981) mentioned that embryogenic callus cultures of date palm were stored at 0, -15, -23, -30 and -196 °C for up three months in the presence of cryoprotective mixture. Revived cultures developed callus, since recovery and development of thawed callus from all frozen temperatures was rapid after a period of 2 - 4 weeks.

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