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Callogenesis, Somatic Embryogenesis and Regeneration of Date Palm *Phoenix dactylifera* L. Cultivars Affected by Carbohydrate Sources

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ABSTRACT

Carbon source could affect the *in vitro* culture of date palm. Little is known about the date palm requirement of sucrose and sorbitol and their concentration. The present study was conducted to determine the effect of sucrose and sorbitol concentrations (0.1, 0.15 or 0.2 mole) and their combinations at all stages of regeneration (callogenesis, proliferation of callus, somatic embryos multiplication, germination and rooting) of three commercial date palm cultivars; Zaghlol, Amry and Malakaby. The results indicated that the *in vitro* response of date palm to carbon source was significantly related to genotype in addition to culture stage. The highest percentages of explants formed callus were achieved using 0.15 M sucrose or sorbitol in induction medium while in proliferation stage, the highest callus fresh weight was observed using 0.1 M sucrose+0.05 M sorbitol. During multiplication and germination stage the highest number of germinated embryos was resulted from 0.05 M sucrose+0.1 M sorbitol, while 0.2 M sucrose enhanced secondary embryo formation. Increasing sucrose concentrations in rooting medium increased significantly number of roots.

Key words: Date palm cultivars, sorbitol, sucrose, embryogenesis, callogenesis, germination

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) an economically important commodity, is a monocotyledonous tree widely cultivated in arid and semi-arid regions (Al-Khateeb, 2006) such as the Middle East and North Africa. Micropropagation, the most promising technology to multiply high-value date palm trees is expected to play an important role in increasing date palm cultivated areas in the recent future (Zivdar *et al*., 2008). Organogenesis and somatic embryogenesis are depending on genotype and hormonal application (El-Bellaj, 2000). Carbohydrates are generally considered as a carbon source needed for growth and development of tissues in *in vitro* cultures. Sucrose is the most frequently used in the culture medium compared with other sources of carbohydrates such as fructose, glucose, sorbitol and mannitol. Several reports show that carbohydrate sources affect the ability of the cells to induce somatic embryogenesis and its development as well as their concentrations (Iraqui and Tremblay, 2001; Tokuhara and Mii, 2003). In several species, sucrose is the preferred carbohydrate not only for induction, proliferation and embryos maturation but playing a role as osmotica (Li *et al*., 1998). Date palm embryogenic mass increased after increasing of sucrose concentration (Zouine and El-Hadrami,

2004). Maturation of soybean somatic embryos can be positively influenced by a low osmotic potential, influenced by carbohydrate concentrations (Walker and Parrott, 2001). A study on conifers showed that increasing the sugar concentration improved the somatic embryo maturation (Iraqui and Tremblay, 2001). In addition, Korbes and Droste (2005) tested different maturation media by using 6% maltose, 3% sucrose or 6% sucrose. Sucrose at 6% significantly enhanced the conversion percent of soybean embryos. Meanwhile, Garin *et al.* (2000) found that a medium with the combination of 88 mM sucrose+175 mM sorbitol was superior to 263 mM sucrose alone, for maturation of *Pinus strobus* somatic embryos.

This study aimed to investigate the effect of sucrose and sorbitol concentrations and their combinations at all stages of regeneration (callogenesis, proliferation of callus, somatic embryos multiplication, germination and rooting) of date palm cultivars; Zaghlol, Amry and Malakaby.

MATERIALS AND METHODS

The present study was performed throughout the period from 2010 to 2012 at the Central Laboratory of Date Palm Research and Development, Agricultural Research Center, Giza, Egypt.

Explant preparation and culture establishment: Off shoots of date palm cultivars; Zaghlol (soft), Amry (semidry) and Malakaby (dry), at 2-4 years-old, were separated from mother trees. Outer leaves were removed, shoot tips were excised and immediately placed in a chilled antioxidant solution consisting of ascorbic and citric acid, 150 mg L^{-1} each (Othmani *et al.*, 2009) to prevent browning. Shoot tips, about 8 cm in length, were surface sterilized in 70% ethanol (one min.) followed by 1.6% w/v sodium hypochlorite (15 min); containing 0.1 mL of tween 20 per 100 mL disinfectant solution. Shoot tips were then rinsed four times with sterile distilled water and placed again in sterile antioxidant solution to prevent shoot tips browning. The tissues surrounding the shoot tips were removed until the primordial leaves were exposed and detached at the base. The shoot tips were sectioned longitudinally into several pieces (Fig. 1a).

Callogenesis: To study the effect of sucrose, sorbitol and its combinations, hundred explants were cultured on MS induction media containing 10 mg L^{-1} 2,4-D+3 mg L^{-1} 2ip+2 g L^{-1} activated charcoal+100 mg L^{-1} glutamine+5 mg L^{-1} thiamine HCl+1 mg L^{-1} biotin (Taha *et al.*, 2007) and 0.1, 0.15, 0.2 mole sucrose, sorbitol at the same concentrations and sucrose+ sorbitol each at 0.05, sucrose+sorbitol at 0.1 and 0.05 mole, sucrose+sorbitol at 0.05 and 0.1 mole, respectively and finally sucrose+sorbitol each at 0.1 mole. All media were solidified with 0.2% Gelrite. The medium pH was adjusted to 5.7- 5.8 prior to autoclaving at 1.4 kg cm^{-2} for 20 min. Cultures were maintained in the dark at 27± 2°C and subcultured every 6 weeks for 9 months under the same culture conditions. After this period, percentages of explants formed callus and amount of callus, estimated visually according to Bottino (1981), were recorded.

Proliferation of callus: After the induction period (9 months) the friable callus obtained in different treatments were separated from the original explants and transferred (0.5 g) to three-quarter strength of MS solidified medium supplemented with 10 mg L^{-1} NAA, 6 mg L^{-1} 2ip (Al-Khayri, 2010), 1.5 g L^{-1} activated charcoal and different concentrations of sucrose, sorbitol and its combinations to proliferate callus. These cultures were maintained for 10 weeks during which they were transferred at 5 weeks interval. Globularization degree, estimated visually (Bottino, 1981), callus fresh weight and percentage of embryo formation were recorded after this period.

Somatic embryos multiplication and germination: Clusters of somatic embryos (3-4 embryos) resulted from previous stages were cultured on half strength MS medium with 0.1 mg L^{-1} NAA+ 0.05 mg L^{-1} BA+ 1.0 g L^{-1} AC and different concentrations and combinations of sucrose and sorbitol for 9 weeks with regular transfer to fresh medium of the same supplements every 3 weeks. Number of secondary embryos and germinated embryos/embryo were recorded.

Rooting stage: Elongated regenerated shoots derived previously (5 cm in length) were cultured on ½ MS rooting medium with different concentrations and combinations of sucrose and sorbitol in addition to 0.2 mg L^{-1} NAA+5 mg L^{-1} thiamine HCl+1 mg L^{-1} Biotin+0.1 mg L^{-1} paclobutrazol solidified with 0.2% Gelrite for 8 weeks (4 weeks interval). Root number per explant and plantlet length (cm) was recorded.

Plant acclimatization: Two- month- old healthy regenerated plantlets with well-developed shoots and roots were removed from rooting medium and the remaining Gelrite was washed under tap water and planted in potting mixture peat moss and perlite (2:1) and cultivated in a green house with 27±2°C under sunlight and 80-90% relative humidity (Fig. 1h).

Statistical analysis: Complete randomized design was adopted with 3 replicates for each treatment. The data were statistically analyzed according to Duncan's multiple range test at 5% level of probability (Steel and Torrie, 1980).

RESULTS

Callogenesis: Table 1 revealed that sucrose at 0.15 M gave the highest average percentage of explants producing callus followed by sorbitol at 0.15 M then sorbitol at 0.20 M while, the lowest percentage appeared with sorbitol at 0.1 M. Zaghlol gave the highest average percentage of explants producing callus followed by Malakaby then Amry. With respect to the interaction, data show that the highest percentage of explants producing callus appeared in Malakaby on sorbitol at 0.20 M while, the highest percentage in Zaghlol appeared with sucrose at 0.10 and 0.15 M. The highest percentage of explants producing callus in Amry appeared with sucrose and sorbitol at 0.1 and 0.05 M, respectively.

Table 2 revealed that the combination of sucrose and sorbitol each at 0.1 M or at 0.10 and 0.05 M in respective order gave the highest amount of callus followed by sucrose at 0.10 M while, the lowest amount of callus was observed at 0.10 M sorbitol. Zaghlol gave the highest amount of callus followed by the two other cultivars. With respect to the interaction, there were insignificant differences between treatments and cultivars.

Proliferation of callus: Data in Table 3 show that the combination of sucrose with sorbitol (0.1 and 0.05 M, respectively) generally gave the highest fresh weight of friable callus followed by (0.1 and 0.1 M) in respective order and sucrose at 0.2 M while, the lowest amount was appeared with sucrose at 0.1 M. With respect to cultivar factor, Zaghlol gave the greatest callus fresh weight followed by Amry then Malakaby. The interaction show that the best result was achieved by Zaghlol with the combination of sucrose and sorbitol (0.10 and 0.05 M, respectively) (Fig. 1b) followed by Amry with sucrose at 0.2 M while, the best result for Malakaby was achieved at 0.15 M sucrose. The lowest result was achieved by Malakaby with sorbitol at 0.15 M.

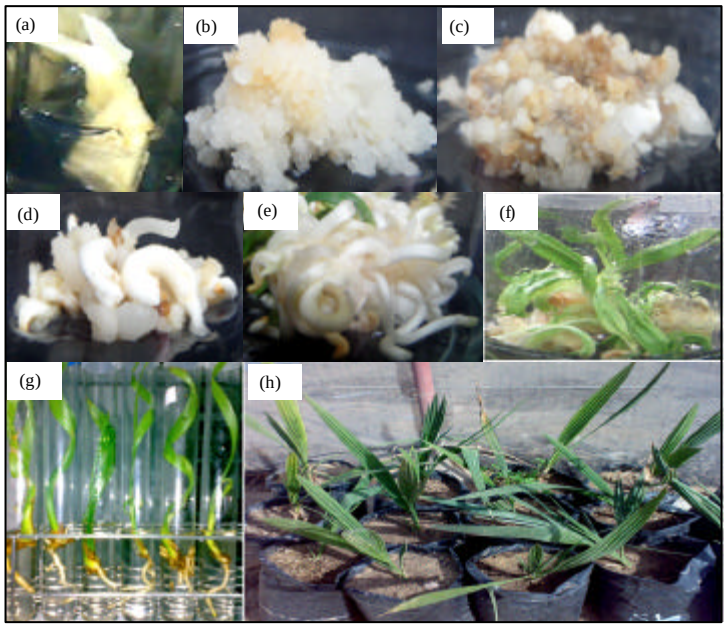

Fig. 1(a-h): Effect of sorbitol and sucrose concentration and their combinations on various stages of *in vitro* culture of date palm (Zaghlol), (a) Shoot tip explant, (b) Callus formation in medium supplemented with 0.1 M sucrose+0.05 M sorbitol, (c) Embryogenic callus at 0.2 M sucrose, (d) Embryo formation at 0.1 M sucrose+0.10 M sorbitol, (e) Embryo multiplication at 0.2 M sucrose, (f) Embryo germination at 0.05 sucrose+0.10 M sorbitol, (g) Rooting with sucrose at 0.15 M, (h) Acclimatization of date palm plants

Table 1: Effect of sucrose, sorbitol and their combinations on percentage of explants producing callus

	Percentage			
	----------Cultivars----------			
Conc. (mole)	Zaghlol	Amry	Malakaby	Mean
Sucrose 0.10	98.33	60.00	95.66	84.66
Sucrose 0.15	98.33	83.30	98.00	93.21
Sucrose 0.20	76.66	66.67	97.66	80.33
Sorbitol 0.10	78.33	43.30	75.00	65.54
Sorbitol 0.15	83.00	95.00	98.66	92.22
Sorbitol 0.20	84.00	72.30	100.00	85.33
Suc 0.05+Sor 0.05	91.00	59.00	50.00	66.67
Suc 0.10+Sor 0.05	92.00	98.33	50.00	80.11
Suc 0.05+Sor 0.10	97.66	85.00	50.00	77.55
Suc 0.10+Sor 0.10	97.66	80.00	75.00	84.22
Mean	89.70	74.29	78.998	

Suc: Sucrose, Sor: Sorbitol

Data in Table 4 show that sucrose at 0.2 M generally gave the greatest degree of globular callus followed by the combination of sucrose with sorbitol each at 0.1 M. while, the lowest degree was appeared with sorbitol at 0.1 M. With respect to cultivar factor, Zaghlol gave the greatest degree of globular callus followed by Amry then Malakaby. The interaction show that the best result

Table 2: Effect of sucrose, sorbitol and their combinations on amount of callus (estimated visually)

	Degree			
	----------Cultivars----------			
Conc. (mole)	Zaghlol	Amry	Malakaby	Mean
Sucrose 0.10	3.66	2.00	2.67	2.78^{b}
Sucrose 0.15	2.67	2.33	2.33	2.44^{d}
Sucrose 0.20	2.33	1.67	3.00	2.11^{e}
Sorbitol 0.10	1.67	1.33	2.00	2.33^{f}
Sorbitol 0.15	2.00	3.00	2.33	2.44^{d}
Sorbitol 0.20	2.33	2.00	3.00	2.44^{d}
Suc 0.05+Sor 0.05	3.00	2.67	2.33	2.67^{c}
Suc 0.10+Sor 0.05	3.33	3.67	1.67	2.89^{a}
Suc 0.05+Sor 0.10	3.66	2.67	1.67	2.67^{c}
Suc 0.10+Sor 0.10	4.33	2.00	2.33	2.89^{a}
Mean	2.898^{a}	2.33^{b}	2.33^{b}	

Means with different letters within each column were significantly different at 5% level, Suc: Sucrose, Sor: Sorbitol

Table 3: Effect of sucrose, sorbitol and their combinations on friable callus fresh weight after three months

	Weight (g)			
	----------Cultivars----------			
Conc. (mole)	Zaghlol	Amry	Malakaby	Mean
Sucrose 0.10	3.70^{hi}	3.35^{jk}	2.21^{no}	3.09^{d}
Sucrose 0.15	4.10^{f}	3.65^{i}	2.56^{m}	3.44^{c}
Sucrose 0.20	3.85^{gh}	5.20^{b}	2.20^{no}	3.75^{b}
Sorbitol 0.10	3.00^{l}	1.95^{pq}	1.50^{s}	2.15^{g}
Sorbitol 0.15	3.20^{k}	2.20^{no}	1.80^{qr}	2.40^{f}
Sorbitol 0.20	3.40^{j}	2.65^{m}	1.70^{r}	2.58^{e}
Suc 0.05+Sor 0.05	4.00^{fg}	3.35^{jk}	2.05^{op}	3.13^{d}
Suc 0.10+Sor 0.05	5.50^{a}	4.75^{d}	2.30^{n}	4.18^{a}
Suc 0.05+Sor 0.10	4.30^{e}	4.00^{fg}	2.10^{op}	3.47^{c}
Suc 0.10+Sor 0.10	5.00^{c}	4.30^{e}	2.20^{no}	3.83^{b}
Mean	4.00^{a}	3.54^{b}	2.06^{c}	

Means with different letters within each column were significantly different at 5% level, Suc: Sucrose, Sor: Sorbitol

Table 4: Effect of sucrose, sorbitol and their combinations on globularization (visually)

	Degree (g)			
	----------Cultivars----------			
Conc. (mole)	Zaghlol	Amry	Malakaby	Mean
Sucrose 0.10	3.00^{g}	3.00^{g}	3.00^{g}	3.00^{e}
Sucrose 0.15	4.33^{c}	3.66^{e}	3.66^{e}	3.88^{c}
Sucrose 0.20	5.00^{a}	5.00^{a}	4.00^{d}	4.67^{a}
Sorbitol 0.10	1.00^{l}	1.00^{l}	1.00^{l}	1.00^{h}
Sorbitol 0.15	1.33^{k}	1.00^{l}	1.00^{l}	1.11^{g}
Sorbitol 0.20	2.00^{j}	1.33^{k}	1.00^{l}	1.44^{f}
Suc 0.05+Sor 0.05	3.66^{e}	2.33^{i}	3.00^{g}	3.00^{e}
Suc 0.10+Sor 0.05	4.00^{d}	4.00^{d}	3.66^{e}	3.89^{c}
Suc 0.05+Sor 0.10	3.33^{f}	2.66^{h}	3.33^{f}	3.11^{d}
Suc 0.10+Sor 0.10	4.66^{b}	4.33^{c}	3.66^{e}	4.22^{b}
Mean	3.23^{a}	2.83^{b}	2.73^{c}	

Means with different letters within each column were significantly different at 5% level, Suc: Sucrose, Sor: Sorbitol

was achieved by Zaghlol (Fig. 1c) and Amry with sucrose at 0.2 M, followed by Zaghlol with the combination of sucrose and sorbitol each at 0.10 M. while, the best result for Malakaby was achieved with sucrose at 0.15 M and the combination of sucrose and sorbitol each at 0.10 M and 0.1 and 0.05, respectively. The lowest result was appeared by the three cultivars with sorbitol at 0.1 M.

Table 5 revealed that sucrose and sorbitol at 0.05 and 0.10 M, respectively, gave the highest average percentage of explants producing embryos followed by sorbitol at 0.20 M while, the lowest percentage appeared with sorbitol at 0.10 M. Amry gave the highest average percentage of explants producing embryos followed by Zaghlol then Malakaby. With respect to the interaction, data show that the highest percentage of explants producing embryos appeared with Amry and sucrose at 0.15 M followed by sorbitol at 0.20 M while, the highest percentage in Zaghlol appeared with sucrose and sorbitol each at 0.10 M (Fig. 1d). The highest percentage of explants producing embryos in Amry appeared with sucrose and sorbitol at 0.05 and 0.10 M., respectively.

Somatic embryos multiplication and germination: Table 6 showed that sucrose at 0.2 M generally gave the greatest number of secondary embryos followed insignificantly by the combination of sucrose with sorbitol each at 0.1 M then (0.1 and 0.05, in respective order) while, the lowest number was appeared with sorbitol at 0.1 M. With respect to cultivar factor, Zaghlol gave the greatest number of secondary embryos followed by Amry then Malakaby. The interaction show that the best result was achieved by Zaghlol with sucrose at 0.2 M (Fig. 1e) and the combination of sucrose with sorbitol at 0.1 and 0.05, in respective order, followed by Amry with sucrose and sorbitol each at 0.1 M while, the best result for Malakaby was achieved with sucrose at 0.2 M and the respective combination of sucrose at 0.1 with sorbitol at 0.05 M or each at 0.1 M, without significant differences. The lowest number of secondary embryos was appeared by Malakaby with sorbitol at 0.1 M.

Table 7 showed that the combination of sucrose with sorbitol (0.05 and 0.10 M) generally gave the best number of converted embryo followed by (0.1 and 0.05 M, in respective order) while, the lowest number was appeared with sorbitol at 0.1 M. With respect to cultivar factor, Zaghlol gave the greatest number of converted embryo followed by Malakaby then Amry. The

Table 5: Effect of sucrose, sorbitol and their combinations on percentage of explants producing embryos

	Percentage			
	----------Cultivars----------			
Conc. (mole)	Zaghlol	Amry	Malakaby	Mean
Sucrose 0.10	18.00	6.00	5.33	9.78
Sucrose 0.15	11.33	41.33	6.00	19.55
Sucrose 0.20	17.33	11.33	5.00	11.22
Sorbitol 0.10	17.00	6.33	4.00	9.11
Sorbitol 0.15	22.67	24.33	5.67	17.56
Sorbitol 0.20	17.33	35.00	8.67	20.33
Suc 0.05+Sor 0.05	10.00	18.33	4.00	10.78
Suc 0.10+Sor 0.05	11.00	24.66	20.33	18.66
Suc 0.05+Sor 0.10	10.33	34.00	27.67	24.00
Suc 0.10+Sor 0.10	27.00	26.00	4.00	19.00
Mean	16.20	22.73	9.07	

Suc: Sucrose, Sor: Sorbitol

Table 6: Effect of sucrose, sorbitol and their combinations on number of secondary embryos

	Embryo No.			
	----------Cultivars----------			
Conc. (mole)	Zaghlol	Amry	Malakaby	Mean
Sucrose 0.10	29.38^{cd}	19.80^{gh}	18.90^{hi}	22.51^{d}
Sucrose 0.15	31.17^{bc}	21.50^{fg}	20.10^{gh}	24.26^{c}
Sucrose 0.20	34.17^{a}	28.20^{d}	26.00^{e}	29.45^{a}
Sorbitol 0.10	15.50^{jk}	11.13^{mn}	5.10^{p}	10.53^{h}
Sorbitol 0.15	18.21^{hi}	14.14^{kl}	7.20^{o}	13.24^{g}
Sorbitol 0.20	21.75^{fg}	12.90^{lm}	10.60^{n}	15.10^{f}
Suc 0.05+Sor 0.05	28.28^{d}	17.00^{ij}	21.30^{ef}	22.20^{d}
Suc 0.10+Sor 0.05	32.71^{ab}	22.40^{f}	25.20^{e}	27.79^{b}
Suc 0.05+Sor 0.10	21.42^{fg}	19.00^{h}	22.50^{f}	21.05^{e}
Suc 0.10+Sor 0.10	29.00^{d}	32.00^{b}	24.50^{e}	28.59^{ab}
Mean	26.43^{a}	19.86^{b}	18.15^{c}	

Means with different letters within each column were significantly different at 5% level, Suc: Sucrose, Sor: Sorbitol

Table 7: Effect of sucrose, sorbitol and their combinations on number of converted embryo

	Embryo No.			
	----------Cultivars----------			
Conc. (mole)	Zaghlol	Amry	Malakaby	Mean
Sucrose 0.10	7.2^{g}	4.8^{k}	3.6^{m}	5.20^{g}
Sucrose 0.15	9.9^{b}	3.0^{n}	3.01^{n}	5.30^{f}
Sucrose 0.20	8.1^{e}	5.4^{j}	2.7^{o}	5.40^{e}
Sorbitol 0.10	5.7^{i}	3.6^{m}	3.6^{m}	4.30^{h}
Sorbitol 0.15	6.5^{h}	5.4^{j}	5.4^{j}	5.77^{c}
Sorbitol 0.20	4.5^{l}	5.4^{j}	7.2^{g}	5.70^{c}
Suc 0.05+Sor 0.05	4.8^{k}	3.6^{m}	7.2^{g}	5.20^{g}
Suc 0.10+Sor 0.05	7.7^{f}	9.72^{c}	5.4^{j}	7.61^{b}
Suc 0.05+Sor 0.10	10.3^{a}	7.6^{f}	9.0^{d}	8.97^{a}
Suc 0.10+Sor 0.10	7.6^{f}	1.8^{p}	7.2^{g}	5.53^{d}
Mean	7.23^{a}	5.03^{c}	5.43^{b}	

Means with different letters within each column were significantly different at 5% level, Suc: Sucrose, Sor: Sorbitol

interaction show that the best result was achieved by Zaghlol (Fig. 1f) with the combination of sucrose and sorbitol (0.05 and 0.1 M, respectively) then sucrose at 0.15 M followed by Amry with sucrose and sorbitol (0.10 and 0.05, respectively) while, the best result for Malakaby was achieved with sucrose and sorbitol (0.05 and 0.10 M, respectively). The lowest result was appeared by Amry with sucrose and sorbitol each at 0.10 M.

Rooting stage: Table 8 showed that sucrose at 0.15 and 0.20 M generally gave the greatest number of roots followed by sucrose at 0.10, the combination of sucrose and sorbitol (0.1 and 0.05 M) and also (0.10 and 0.10) in respective order. All sorbitol concentrations inhibited root formation especially at 0.10 M which gave no roots for all cultivars. With respect to cultivar factor, Zaghlol gave the greatest number of roots followed by Amry then Malakaby. The interaction show that the best result was achieved by Zaghlol with sucrose at 0.15 M (Fig. 1g) followed by 0.20 M then 0.10 M while, the best results for Amry and Malakaby were achieved with sucrose at 0.20 M. The inhibition of rooting appeared in the three cultivars when treated with sorbitol at 0.10 M and

Table 8: Effect of sucrose, sorbitol and their combinations on root number

	Root No.			
	----------Cultivars----------			
Conc. (mole)	Zaghlol	Amry	Malakaby	Mean
Sucrose 0.10	5.10^{c}	2.00^{hijk}	1.00^{klmn}	2.70^{b}
Sucrose 0.15	8.30^{a}	4.00^{def}	1.66^{ijkl}	4.65^{a}
Sucrose 0.20	6.50^{b}	5.00^{cd}	2.33^{hij}	4.61^{a}
Sorbitol 0.10	0.00^{n}	0.00^{n}	0.00^{n}	0.00^{f}
Sorbitol 0.15	0.33^{mn}	0.00^{n}	0.00^{n}	0.11^{f}
Sorbitol 0.20	0.88^{lmn}	0.00^{n}	0.66^{lmn}	0.51^{ef}
Suc 0.05+Sor 0.05	2.50^{ghi}	0.66^{lmn}	0.00^{n}	1.05^{de}
Suc 0.10+Sor 0.05	4.40^{cde}	2.00^{hijk}	1.66^{ijkl}	2.68^{b}
Suc 0.05+Sor 0.10	3.00^{fgh}	1.00^{klmn}	0.66^{lmn}	1.55^{cd}
Suc 0.10+Sor 0.10	3.50^{efg}	1.66^{ijkl}	1.33^{jklm}	2.16^{bc}
Mean	3.45^{a}	1.63^{b}	0.931^{c}	

Means with different letters within each column were significantly different at 5% level, Suc: Sucrose, Sor: Sorbitol

Table 9: Effect of sucrose, sorbitol and their combinations on plantlet length

	Length (cm)			
	----------Cultivars----------			
Conc. (mole)	Zaghlol	Amry	Malakaby	Mean
Sucrose 0.10	11.40^{cde}	12.0^{cd}	10.0^{efgh}	11.13^{bc}
Sucrose 0.15	11.30^{cde}	10.8^{defg}	9.5^{fghi}	10.53^{c}
Sucrose 0.20	9.70^{fgh}	8.5^{hij}	9.0^{ghij}	9.07^{d}
Sorbitol 0.10	7.70^{j}	7.5^{j}	8.0^{ij}	7.73^{e}
Sorbitol 0.15	8.50^{hij}	8.0^{ij}	8.5^{hij}	8.33^{de}
Sorbitol 0.20	8.97^{hij}	8.5^{hij}	8.8^{hij}	8.76^{d}
Suc 0.05+Sor 0.05	13.83^{ab}	9.0^{ghij}	8.0^{ij}	10.28^{c}
Suc 0.10+Sor 0.05	14.40^{a}	13.6^{ab}	9.5^{fghi}	12.50^{a}
Suc 0.05+Sor 0.10	11.93^{cd}	12.5^{bc}	10.0^{efgh}	11.48^{b}
Suc 0.10+Sor 0.10	13.90^{ab}	11.0^{cdef}	10.5^{defg}	11.80^{ab}
Mean	11.16^{a}	10.14^{b}	9.18^{c}	

Means with different letters within each column were significantly different at 5% level, Suc: Sucrose, Sor: Sorbitol

by Amry and Malakaby with sorbitol at 0.15 M and also Amry with sorbitol at 0.20 M. Figure 1h presents acclimatization of date palm plants.

Table 9 showed that the combination of sucrose with sorbitol (0.10 and 0.05 M, in respective order) generally gave the greatest plantlet length followed by their combination at 0.1 M for each while; the lowest length was appeared with sorbitol at 0.1 M. With respect to cultivar factor, Zaghlol gave the greatest plantlet length followed by Amry then Malakaby. The interaction show that the best result was achieved by Zaghlol with the combination of sucrose and sorbitol (0.10 and 0.05 M), (0.05 and 0.05 M) and (0.10 and 0.10 M) in respective order. Amry gave the best length of its plantlets with sucrose and sorbitol (0.10 and 0.05 M, respectively), while the best result for Malakaby was achieved with sucrose at 0.10+sorbitol at 0.10 M. The lowest result was achieved by the three cultivars with sorbitol at 0.10 M.

DISCUSSION

The highest percentages of explants formed callus were obtained when using 0.15 M sucrose or sorbitol at the same concentration in induction medium, while in proliferation stage, the highest

callus fresh weight was achieved using 0.1 M sucrose +0.05 M sorbitol. This result could be explained by Brhadda *et al.* (2008). It was found that the plasmolysis of cells may stimulate cell division activity and increase somatic embryos production. Meanwhile, No morphogenesis with sorbitol was obtained in olive calli or somatic embryogenesis compared with mannitol, saccharose and different saccharose concentrations tested. Wetherell (1984) described that the embryogenic effect of 0.1 M sucrose or sorbitol pre-treatments is due to isolation of callus cells by plasmolysis with subsequent rupturing plasmodesmata. Meanwhile, Bronsema *et al.* (1997) assured that immature maize zygotic embryos produced fewer callus on sorbitol than on sucrose containing medium.

Sucrose and sorbitol at 0.05 and 0.10 M, respectively, gave the highest average percentage of explants producing embryos. This result agreed with the findings of Kavi Kishor (1987) which indicated that sorbitol or manitol in combination with sucrose was found beneficial for inducing differentiation in long term culture in rice, compared with the control which gave the lowest result. Sucrose greatly enhanced embryos multiplication followed insignificantly by its combination with sorbitol (0.1 M for each) or (0.1 and 0.05, in respective order). This result could be in harmony with the findings of Yadollahi *et al.* (2011). The enhancement of the secondary embryo percentage and mean number of secondary embryo per rapeseed microspore-derived embryo could be observed with the application of sorbitol or glucose in induction medium. Oil palm secondary somatic embryos were achieved on MS medium supplemented with 0.2 M of sorbitol and 200 mg L^{-1} ascorbic acid (Sanputawong and Te-chato, 2011). Also, Te-chato and Hilae (2007) showed that among various carbon sources tested, the use of 0.2 M sorbitol in culture medium resulted in the best secondary somatic embryos (SSE) induction either in percentage (100 primary haustorium embryos, PHE) or number of induced SSE (21.55 SSE/PHE) for oil palm. Garin *et al.* (2000) found that a medium with 88 mM sucrose plus 175 mM sorbitol solidified with 1.0% gellan gum produced high number of somatic embryos in four out of five embryogenic lines tested of *Pinus strobes*. Mannitol treatments gave lower results than sorbitol and fructose. It has been observed that high concentrations of carbohydrates can create osmotic stress-improving embryogenesis (Agarwal *et al.*, 2004).

The presence of sorbitol with sucrose as a source of carbohydrates proved to be important for embryo germination (conversion). This result was agreed with the finding of Kunitake *et al.* (1993) in *Rosa rugosa* Thunb. After transferring the embryos (1-2 mm) to MS medium containing 0.1 M sorbitol, as the sole carbon source, 3% of them germinated and grew into plantlets which showed sustained growth. Samoylov *et al.* (1998) indicated that sucrose promotes faster embryos histo-differentiation and maturation and allows the recovery of up to 50% more mature, cotyledon stage embryos within three weeks. The sucrose concentration in the medium significantly affected somatic embryo germination. The best rates were obtained between 90 and 450 mM sucrose. High sucrose concentration (630 mM) was shown to hamber germination (Mauri and Manzanera, 2004). Iraqui and Tremblay (2001) stated that embryos matured on 6% sucrose contained significantly more soluble and insoluble proteins than embryos matured on any other treatments. Also, a maturation medium of black spruce and white spruce somatic embryos containing 6% sucrose, gave significantly more embryos than a medium containing 3.16% of each glucose and fructose.

From this investigation we can conclude that it could be important to adjust the source of carbohydrate and its concentration at every stage of regeneration of date palm cultivars. Kunitake *et al.* (1993) stated that callus of *Rosa rugosa* Thunb. were subcultured at 20-day intervals on MS medium containing 0.1-0.2 M galactose on which they grew rapidly; but somatic embryogenesis was inhibited. Somatic embryos were again induced from the subcultured calli after

transferring to MS medium containing 0.1 M fructose or sucrose but lacking growth regulators. Meanwhile, Gonzalez *et al.* (2001) found that maltose was superior to sucrose in terms of the number of *Abies nordmanniana* somatic embryos formed, as well as their germination percentage; the mixture of maltose and sucrose were not found to produce as good results as pure maltose.

The different responses of date palm cultures to carbohydrate sources and their concentrations could be explained. It seems that, sucrose followed by hydrolysis throughout the maturation period may be a regulator factor for the synthesis of storage proteins and sugars in date palm somatic embryos (Zouine and El-Hadrami, 2004). Iraqui and Tremblay (2001) stated that embryos matured on 6% sucrose which gave significantly more embryos than a medium containing 3.16 % of each glucose and fructose contained significantly more soluble and insoluble proteins than embryos matured on the other treatments.

This investigation also revealed that date palm cultivars could differ in response to the source of carbohydrates. This result was agreed with Zouine and El-Hadrami (2004). They proved that the effect of sucrose on cell suspension culture of date palm depends on cultivar. Also Garin *et al.* (2000) assured that the effect of sucrose concentration on the maturation of somatic embryos of *Pinus strobus* depended on genotype. Sucrose at 263 mM and 350 mM increased the number of mature somatic embryos production of line SY4, while reducing that of line SAA26 compared with sucrose at 88 mM. In addition, the effects of carbon sources were evaluated on secondary embryo induction and maturation in rapeseed microspore-derived embryos of cultivars Global, PF704 and Option. Among various carbon sources tested (sucrose, glucose, fructose and sorbitol), the use of 0.3 M glucose and 0.2 M sorbitol in secondary embryo induction medium (for cultivars Global and PF704) and sorbitol at 0.2 and 0.3 M (for cultivar Option), induced the highest secondary embryogenesis percentage. The highest number of secondary embryos was observed with 0.2 M sorbitol in cv. Global and with 0.3 M glucose in cvs. PF704 and Option (Yadollahi *et al.*, 2011).

Sucrose proved to be the best source of carbohydrates for rooting. This result is agreed with the findings of Pawlicki and Welander (1995). Discs cut from micropropagated axillary shoots of the apple rootstock Jork 9 were used to study adventitious rooting *in vitro*. Different types, concentrations and combinations of carbohydrates were investigated to identify the optimal conditions for root differentiation. These studies demonstrated that sucrose (29-59 mM) promoted root formation but also callus formation. Sorbitol, on the other hand, required higher concentrations for optimum rooting but reduced callus formation. Combination of glucose and sorbitol (117/59 mM) or sucrose and mannitol (5929 mM) resulted in 100% root formation of cultured discs with more than six roots per disc. Also, with the combination sucrose/mannitol, the time required for optimum rooting was reduced compared to other combinations. Correa *et al.* (2005) stated that comparisons between related species with different rooting capacities can provide insights into the mechanisms controlling adventitious root development. The availability of carbohydrates is often considered exclusively as an energetic requirement to drive root development; the major regulatory role in the process is often attributed to phytohormones, particularly auxin. The roles of light quantity (irradiance) and carbohydrate supply available to young aseptic donor-plants on the adventitious rooting response of *Eucalyptus globulus* (rooting recalcitrant) and *E. saligna* (easy-to-root) were examined. In *E. globulus*, rooting was promoted by the absence of sucrose in donor-plant media. Presence of sucrose in donor plant medium promoted root number but did not affect rooting percentage of *E. saligna*. A positive effect of glucose on cutting rhizogenesis was found if this hexose was supplied during the root induction phase, followed by sucrose in the root formation step, especially for *E. globulus*. The same effect was not seen with fructose.

It could be assured that the combination of sucrose and sorbitol could largely affect plant length, at rooting stage, as it gave the highest plantlet length of date palm cultivars under investigation while, sucrose gave a satisfactory length. However, Garcia-Martin *et al.* (2001) claimed that high sucrose concentration seemed to improve shoot elongation of cork somatic embryos and best results on plant conversion were obtained with 50 g L^{-1}.

CONCLUSION

The results indicated that the *in vitro* response of date palm cultures to carbon source was significantly related to genotype. The effective type of carbon is differed according to the stage of date palm regeneration. The presence of sorbitol with sucrose as a source of carbohydrates proved to be important for proliferation of the callus, embryo multiplication and germination. Meanwhile, sucrose proved to be the best carbon source for date palm rooting.

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