IN VITRO CONSERVATION OF DATE PALM SHOOT TIP EXPLANTS UNDER MINIMAL GROWTH CONDITION

EL-DAWAYATI, MAIADA M.[1], EL- I. BAKER[2,] AMINA H. GOMAA[2] AND ZEINAB E. ZAYED[1]

1. *The Central Laboratory for Date Palm Researches and Development, ARC, Giza, Egypt. Maiada_dw@hotmail.com*
2. *Department of Pomology, Faculty of Agriculture, Cairo University, Egypt.*

(Manuscript received 21 May 2012)

Abstract

This current investigation was conducted to study the effect of different sugar type (sucrose, sorbitol or mannitol at 0.3M) with different ABA concentration (0.0, 2.0, 4.0, 6.0 and 8.0 mg/l) on the shoot tip explants of date palm Zaghlool cv. conserved at 5 or 15 C° under complete darkness for six and 12 months by observing the physiological changes and growth degree of the conserved explants during the conservation period and also their recovery when they were returned and recultured under normal growth condition in order to achieve the best minimal growth condition for in vitro conservation of shoot tip explants. All shoot tip explants conserved at 5 C° for six month or at 15 C° for 12 months on conservation medium supplemented with 0.3M sucrose or sorbitol combined with different concentration of ABA able to survive.

Yet, there are few information about conservation of date palm shoot tip explants or embryonic callus by in vitro slow growth storage so this approach needs more studies to keep continuously viable sterilized and contaminated free new source from mother plants for labor commercial production.

Key words: Date palm, Conservation, Minimal growth condition, Sugar, ABA

INTRODUCTION

In tropical and subtropical regions, palms are well represented among cultivated species. Date palm, coconut palm, and oil palm are among the most important crops in the economy of many developing countries around the world. Date palm (*Phonix dactylifera* L.) is considered one of the most important commercial crops in the Arab worlds.

The safe conservation of genetic resources of palm species is faced with various problems Indeed, germplasm collections of these species are conserved under field conditions since seeds of oil palm and coconut are recalcitrant and those of date palm have a germination rate which decreases rapidly even after short storage periods (Engelmann *et. al.,* 1995). Tissue culture provides new methods for storing the plant material needed for many purpose such as delayed planting until climatic conditions are again favorable, conserve stocks of horticultural and agriculturally interesting species or varieties, retain genotypes for as long as they are needed in immediate plant breeding programs and preserve the widest possible use in future

(George, 1993). There have been two approaches to the storage of vegetatively propagated germplasm. In one, the aim is to reduce the growth rate of the cells or plants by the manipulations of culture media, reduction of incubation temperature and the combination of the former two methods. Another approaches is to stop the growth altogether (Benson, 1994). The advantage of this technology is that a variety of cells and tissue can be stored: for instance protoplasts, single cells and organized tissues, for example, meristems and somatic embryos (Bajaj, 1986). The principle of reduced-growth storage is based on the manipulations of culture conditions/culture media to allow the cultures to remain viable, but with a growth rate that is very slow. The main advantages of this method are that culture deterioration can be detected visually and there fore loss of viability can be avoided, cultivars can be pathogene tested, so materials will be available for international (Ng and Ng, 1991). Very little research has so far been carried out on the germ-plasm storage of dates. The current investigation was conducted to study the effect of these different sugar (sucrose, sorbitol or mannitol at 0.3M) with five different ABA concentration at (0.0, 2.0, 4.0, 6.0 and 8.0 mg/l) on the shoot tip explants of date palm Zaghlool cv. conserved at 5 or 15 C° under complete darkness for six and 12 months by observing the physiological changes and growth degree of the conserved explants during the conservation period and also their recovery when they were returned and recultured under normal growth condition

MATRIALS AND METHODS

The following experiment was aimed to study the *in vitr*o conservation of date palm shoot tip explants under different minimal growth conditions and to examine the recovery of the conserved explants when they were returned and recultured on normal growth medium and incubated under normal growth conditions in order to achieve the best minimal growth conditions for *in vitro* conservation of date palm explants.

Effect of different sugar and abscisic acid (ABA) concentrations on shoot tip explants of date palm Zaghlool cv. conserved at 5°C or 15 °C under complete darkness for 6 and 12 months

In this experiment uncontaminated shoot tip explants of Zaghlool cv. which established on starting medium consists of MS basal nutrient medium (1962) + 3.0 mg/L 2iP + 10.0 g/L 2, 4 – D + 3.0 g/L activated charcoal. The explants were transferred on conservation media which consists of starting medium+ different sugar types (sucrose, sorbitol or Mannitol) each at 0.3 M\L different abscisic acid (ABA) concentrations at 2.0, 4.0, 6.0 and 8.0 mg/L and ABA- free conservation medium was also investigated.

The pH of each medium was adjusted to 5.7 ± 0.1 prior to addition of 8.0 g/L agar. The medium was distributed into culture jars (150 mL) where each one contained 40 mL. The culture jars were immediately capped with polypropylin closure

and then the medium was sterilized by autoclaving at 121°C and 15 Ibs/in^2 for 20 min.

Each treatment were represented by three replicates each of 3 culture jars and each jar contained one longitudinal section of shoot tip explants.

The culture jars of each treatment were divided into two equal groups the first group conserved at 5°C under complete darkness, and the second group conserved at 15°C under complete darkness

Data were calculated after 6 months and after 12 months about the following changes on the conserved explants to indicate

1. The average degree value of callus initiation /explant.
   These data were scored visually according to Pottino (1981)
2. Biochemical analysis: data were taken at the end of conservation (after 12 month) to record the changes in total soluble sugar content, reduced sugar content and proline amino acid content which affected by stress conditions.

Total soluble sugars were determined in isopropanol extract by using the phenol - sulphuric method according to Dubois *et. al.,* (1956).

Reducing sugars were determined in ethanolic extract, using phosphomolybdic method A . O . A . C . (1980).

3. To determine Proline according to the method recorded by Bates, *et. al.,* (1973).
4. Survival percentage= $\frac{\text{Number of live explants}}{\text{The total number of explants}} \times 100$

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 Snedecor and Cochran (1972).

RESULTS AND DISCUSSION

a. Effect of different sugar and different ABA concentrations on shoot tip explants of Zaghlool cv. conserved at 5°C under complete darkness for 6 and 12 months

1. Callus initiation degree value

Data in Table (1) showed that callus initiation from conserved shoot tip explants didn't affect significantly by different sugar type or different ABA concentration added to conservation media and also by different conservation period (6and12 months).

Table1. Effect of different sugar and different ABA concentrations on the callus degree value initiated from shoot tip explants of Zaghlool cv. conserved at 5°C under dark for 6 and 12 months.

(A) Sugar 0.3 M	(B) ABA mg/L	(C) Conservation Period (month)		
		6	12	(A B) Mean
Sucrose	0.0	1.00	1.33	1.16
	2.0	1.11	1.33	1.22
	4.0	1.11	1.33	1.22
	6.0	1.11	1.11	1.11
	8.0	1.11	1.11	1.11
Mean (A)		1.08	1.24	1.16
Sorbitol	0.0	1.00	1.11	1.05
	2.0	1.22	1.22	1.22
	4.0	1.11	1.66	1.38
	6.0	1.11	1.44	1.27
	8.0	1.33	1.44	1.38
Mean (A)		1.15	1.37	1.26
Mannitol	0.0	1.00	1.22	1.11
	2.0	1.00	1.44	1.22
	4.0	1.00	1.11	1.05
	6.0	1.00	1.33	1.16
	8.0	1.00	1.00	1.00
Mean (A)		1.00	1.22	1.10
Mean (C)		1.08	1.27	

(B) ABA mg/L

0.0	2.0	4.0	6.0	8.0
1.11	1.22	1.22	1.18	1.16

(B) ABA mg/L	(C) Conservation Period (month)	
	6	12
0.0	1.00	1.22
2.0	1.11	1.33
4.0	1.07	1.36
6.0	1.07	1.29
8.0	1.14	1.18

L.S.D $_{0.05}$ for

A	N.S	AB	0.30
B	N.S	AC	N.S
C	N.S	BC	N.S
		ABC	N.S

* Values determined as described by Pottino (1981).

The interaction between the effect of different sugar type (Sucrose, sorbitol, mannitol) and different ABA concentration added to conservation media on the callus

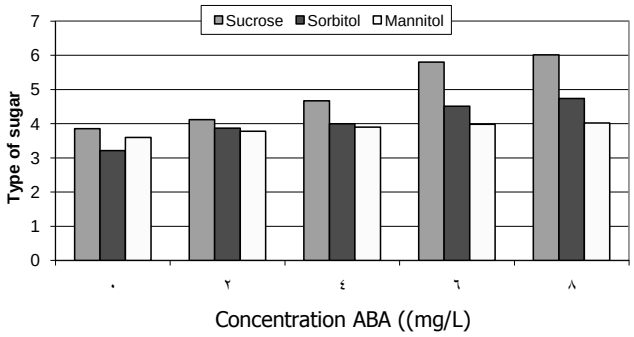
initiated from the shoot tip explants recorded significant effect , data indicated that all shoot tip explants conserved on conservation medium supplemented with 2.0 mg/L ABA combined with sucrose, sorbitol or mannitol gave the same degree value of callus initiation (1.22). Conservation media supplemented with sorbitol and ABA at 4.0 mg/L or 8.0 mg/L gave the highest significant degree value of callus initiation as showed the same degree value (1.38).

2. Total soluble sugar content

Data in Figure (1) showed that the content of total soluble sugar of shoot tip explants conserved at 5°C for 12 months affected significantly with the addition of different sugar type to the conservation media, with sucrose addition to the conservation media the highest significant of total soluble sugar content was achieved while the addition of mannitol to the conservation media the lowest significant of total soluble sugar was achieved.

Different ABA concentrations added to conservation media recorded significant differences on the total soluble sugar content of shoot tip explants. Conserved shoot tip explants on ABA- free conservation medium gave the lowest significant mean value of total soluble sugar content on other hand , conserved shoot tip explants on conservation medium supplemented with ABA at 6.0 or 8.0mg/L gave the highest significant of total soluble sugar content.

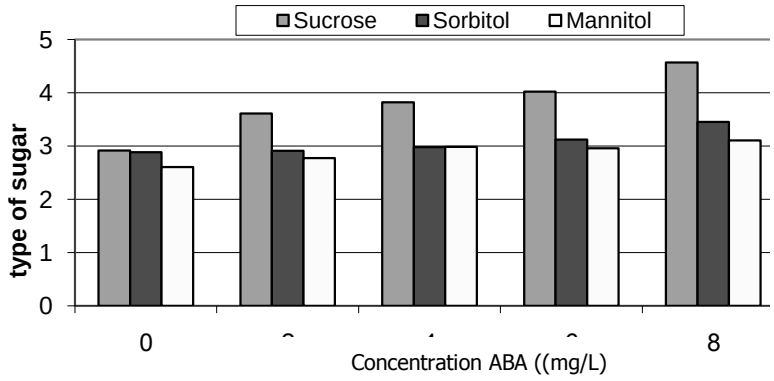
Figure 1. Effect of different sugar and different ABA concentrations on total soluble sugar content (mg\100 f.wt.) of Zaghlool cv shoot tip explants conserved at 5°C under dark for 12 months



3. Reduced sugar content

Data in Figure (2) indicated that different sugar type which added to conservation medium recorded significant differences on reduced sugar content of shoot tip explants. Data clearly showed that added sucrose to conservation media resulted the highest significant reduced sugar content followed significantly by the reduced sugar content of shoot tip explants conserved on media supplemented with sorbitol while the lowest significant reduced sugar content obtained when shoot tip explants conserved on media supplemented with mannitol.

Figure 2. Effect of different sugar and different ABA concentrations on reduced sugar content (mg\100 f.wt.) of Zaghlool cv. shoot tip explants conserved at 5°C under dark for 6and12 months



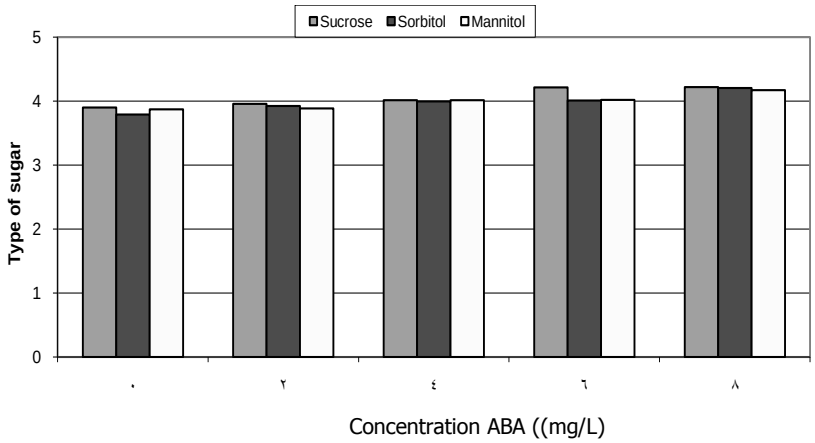
Data clearly showed that different ABA concentrations which added to conservation media affected significantly the reduced sugar content of shoot tip explants, the highest reduced sugar content was achieved when shoot tip explants conserved on medium supplemented with ABA at 8.0mg/L while, shoot tip explants conserved on ABA-free medium resulted the lowest significant reduced sugar content of shoot tip explants.

4. Prolin amino acid content

Data in Figure (3) showed that the addition of different sugar type to conservation media didn't affect significantly the prolin amino acid content of shoot tip explants. While the addition of different ABA concentrations to the conservation media affected significantly the prolin amino acid content of shoot tip explants.

Data clearly showed that increasing ABA concentration on conservation media increased significantly the proline amino acid content of shoot tip explants The lowest significant of proline amino acid content was obtained when shoot tip explants conserved on ABA- free conservation medium and medium supplemented with 2.0 mg/L ABAwithout significant difference in between. while, the highest significant of prolin amino acid content was obtained when shoot tip explants conserved on medium supplemented with 8.0 mg/L ABA.

Figure 3. Effect of different sugar and different ABA concentrations on Prolin amino acid content (mg\100 f.wt.) of Zaghlool cv. shoot tip explants conserved at 5°C under dark for 6and12 months.



5. Survival percentage

Data in Table (2) clearly revealed the significant effect of different sugar type added to conservation medium on the survival percentage of the conserved shoot tip explants when returned to recultured on recovery medium for 4 weeks after conservation period, conserved shoot tip explants on conservation medium supplemented with sucrose or sorbitol recorded the highest significant survival percentage (78.88%, 77.77% respectively) where the lowest significant for survival percentage was obtained when shoot tip conserved on conservation medium supplemented with mannitol (41.10.1%).

Referring to the effect of different ABA concentration added to conservation media data revealed that there were no significant differences among different treatment on the survival percentage of conserved shoot tip explants when recultured on normal growth medium and incubated under normal growth conditions for 4 weeks. In the respect of the effect of conservation period (6 or 12 months) on the survival percentage data showed that 86.66% of the shoot tip explants conserved for 6 months can able to survive when returned to reculture on normal growth medium and incubated under normal growth conditions for 4 weeks. This percentage reduced significantly to 45.17% when shoot tip explants conserved for 12 months.

The interaction between the effect of different sugar type added to conservation media and the conservation period (6and 12 months) on the survival percentage of shoot tip explants recorded significant differences.

All shoot tip explants conserved for 6 months on media supplemented with sucrose or sorbitol able to survive completely (100%) while this survival percentage decreased significantly to 59.99 % when shoot tip explants conserved for 6 months on media supplemented with mannitol

Table 2. Effect of different sugar and different ABA concentrations on survival percentage of Zaghlool cv. shoot tip explants conserved at 5°C under dark for 6and12 months. (when recultred on normal growth medium and incubated under normal growth conditions for 4 weeks)

(A) Sugar 0.3 M	(B) ABA Mg/L	(C) Conservation Period (month)		
		6	12	(AB) Mean
Sucrose	0.0	100.00	55.55	77.77
	2.0	100.00	66.66	83.33
	4.0	100.00	66.66	83.33
	6.0	100.00	44.44	72.22
	8.0	100.00	55.55	77.77
Mean (A)		100.00	57.77	78.88 a
Sorbitol	0.0	100.00	66.66	83.33
	2.0	100.00	55.55	77.77
	4.0	100.00	44.44	72.22
	6.0	100.00	55.55	77.77
	8.0	100.00	55.55	77.77
Mean (A)		100.00	55.55	77.77 a
Mannitol	0.0	77.77	22.22	74.99
	2.0	66.66	33.33	49.99
	4.0	44.44	33.33	83.88
	6.0	55.55	11.11	33.33
	8.0	55.55	1.11	33.33
Mean (A)		59.99	22.22	41.15 b
Mean (C)		86.66 a	45.17 b	

(B) ABA				
0.0	2.0	4.0	6.0	8.0
70.36	70.36	64.81	61.10	62.96

(B) ABA mg/L	(C) Conservation Period (month)	
	6	12
0.0	92.59	48.14
2.0	88.88	51.84
4.0	81.48	48.14
6.0	85.18	37.03
8.0	85.18	40.73

L.S.D $_{0.05}$ for

A	11.47	AB	N.S
B	N.S	AC	6.84
C	7.25	BC	N.S
		ABC	N.S

Increasing the conservation period of shoot tip explants to 12 months decreased the survival percentages compared with those conserved for 6 months as the survival percentage of shoot tip explants conserved on media supplemented with sucrose decreased from 100% to 57.77%, survival percentage of shoot tip explants conserved on media supplemented with sorbitol decreased from 100% to 55.55% and the survival percentage of shoot tip explants conserved on media supplemented with mannitol decreased from 59.99 to 22.22%.

b. Effect of different sugar and different ABA concentrations on shoot tip explants of date palm Zaghlool cv. conserved at 15°C under dark for 6 months and 12 months.

1. Callus initiation degree value

Data in Table (3) showed that the degree values of callus initiated from shoot tip explants were affected significantly by the addition of different sugar type to conservation media. The lowest significant degree value of callus initiated from shoot tip explants was obtained when sucrose was added to conservation medium as shoot tip explants can not able to initiate any callus while the highest significant degree value of callus initiation was achieved from shoot tip explants conserved on medium supplemented with sorbitol (1.54) followed significantly by the degree value of callus initiated from shoot tip explants conserved on medium supplemented with mannitol (1.04).

Regarding to the effect of different ABA concentrations added to medium, the highest significant degree value of callus initiation was achieved when the shoot tip explants conserved on medium supplemented with ABA at 2.0 and 4.0mg/L (1.42 and 1.29 respectively) without significant difference in between. Followed significantly by the degree values of callus initiated from shoot tip explants conserved on ABA- free medium and media supplemented with 8.0 or 6.0mg/L ABA (1.12, 1.12 and 1.01 respectively) without significant differences in between

Investigation about the effect of conservation period (6 and 12 months) on the callus initiated from shoot tip explants showed that increasing the conservation period from 6 months to 12 months didn't affect significantly the callus initiation.

Table 3. Effect of different sugar and different ABA concentrations on the callus degree value initiated from shoot tip explants of Zaghlool cv. conserved at 15°C under dark for 6 and 12 months.

(A) Sugar 0.3 M	(B) ABA Mg/L	(C) Conservation Period (month)		
		6	12	(AB) Mean
Sucrose	0.0	1.00	1.00	1.00
	2.0	1.00	1.00	1.00
	4.0	1.00	1.00	1.00
	6.0	1.00	1.00	1.00
	8.0	1.00	1.00	1.00
Mean (A)		1.00	1.00	1.00 c
Sorbitol	0.0	1.11	1.44	1.27
	2.0	2.33	2.22	2.27
	4.0	1.66	1.88	1.77
	6.0	1.00	1.11	1.05
	8.0	1.11	1.66	1.38
Mean (A)		1.44	1.66	1.54 a
Mannitol	0.0	1.00	1.22	1.11
	2.0	1.00	1.00	1.00
	4.0	1.00	1.22	1.11
	6.0	1.00	1.00	1.00
	8.0	1.00	1.00	1.00
Mean (A)		1.00	1.08	1.04 b
Mean (C)		1.14	1.25	

(B) ABA mg/L				
0.0	2.0	4.0	6.0	8.0
1.12 b	1.42 a	1.29 a	1.01 b	1.12 b

(B) ABA mg/L	(C) Conservation Period (month)	
	6	12
0.0	1.03	1.22
2.0	1.44	1.40
4.0	1.22	1.36
6.0	1.00	1.03
8.0	1.03	1.22
L.S.D $_{0.05}$ for		
A 0.12	AB 0.28	
B 0.16	AC N.S	
C N.S	BC N.S	
	ABC N.S	

* Values determined as described by Pottino (1981).

Data about the interaction between the effect of different sugar type and different ABA concentrations added to conservation medium showed significant effect on degree values of callus initiated from the shoot tip explants. All shoot tip explants conserved on media supplemented with sucrose and different ABA concentrations can not able to initiated callus. With sorbitol the highest significant degree value of callus initiation was produced from medium supplemented with 2.0 mg/L ABA (2.27) followed significantly by the degree value of callus initiated from shoot tip explants conserved on conservation medium supplemented with 4.0 mg/L ABA (1.77), then the

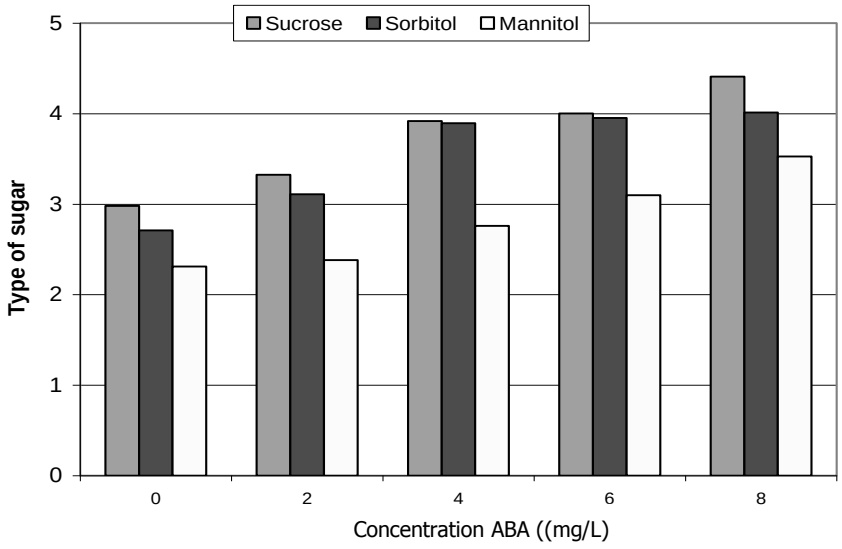
degree value of callus initiated from shoot tip explants conserved on medium supplemented with 8.0 mg/L ABA (1.38), while the lowest significant degree value of callus initiation was obtained from shoot tip explants conserved on conservation medium supplemented with 6.0 mg/L ABA (1.05).

Concerning to the effect of mannitol sugar combined with different ABA concentration data showed that shoot tip explants conserved on medium supplemented with mannitol and ABA at 2.0, 6.0 or 8.0mg/L failed completely to initiate any callus while, shoot tip explants conserved on medium supplemented with mannitol and ABA at 4.0 mg/L and ABA- free medium able to initiated callus with low degree value (1.11).

2. Total soluble sugar content

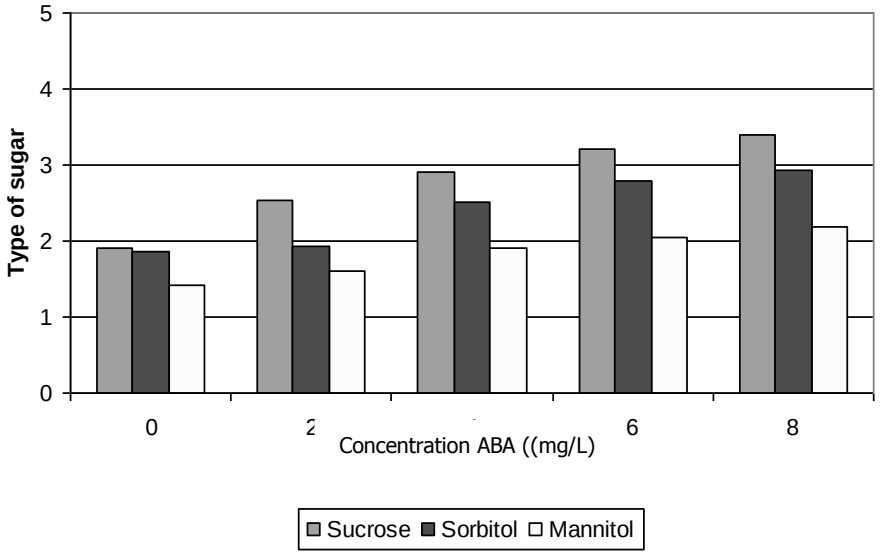
Data in Figure (4) showed that different sugar type added to conservation media had a significant effect on total soluble sugar content of shoot tip explants.

Figure 4. Effect of different sugar and different ABA concentrations on total soluble sugar content (mg\100 f.wt.) of Zaghlool cv shoot tip explants conserved at 15°C under dark for 6and12 months.



Referring to the effect of different ABA concentrations on total soluble sugar content of shoot tip explants, it was clearly observed from the results that increasing ABA concentration increased significantly the total soluble sugar content of shoot tip explants. ABA- free conservation medium recorded the lowest significant total soluble sugar content. While medium supplemented with 8.0mg/L ABA recorded the highest significant total soluble sugar content followed significantly by the total soluble sugar obtained from shoot tip explants conserved on medium supplemented with 6.0 or 4.0mg/L ABA without significant difference in between.

Figure 5. Effect of different sugar and different ABA concentrations on reduced sugar content (mg\100 f.wt.) of Zaghlool cv shoot tip explants conserved at 15°C under dark for 6 and 12 months.



3. Reduced sugar content

Data in Figure (5) clearly revealed that different sugar type showed significant effect on reduced sugar content of conserved shoot tip explants. The highest significant reduced sugar content was showed when shoot tip explants conserved on medium supplemented with sucrose but with the addition of mannitol to conservation medium the lowest significant reduced sugar content of shoot tip explants was obtained while, reduced sugar content of shoot tip explants conserved on media supplemented with sorbitol came significantly in between.

Regarding to the effect of different ABA concentration added to conservation medium on reduced sugar content of shoot tip explants the highest significant reduced sugar content of shoot tip explants was achieved by conserved explants on medium supplemented with 8.0 mg/L ABA. This followed significantly in descending order by conserved shoot tip explants on media supplemented with 6.0, 4.0or 2.0mg/L ABA with significant differences in between while this result reduced significantly to the lowest significant when shoot tip explants conserved on ABA- free medium.

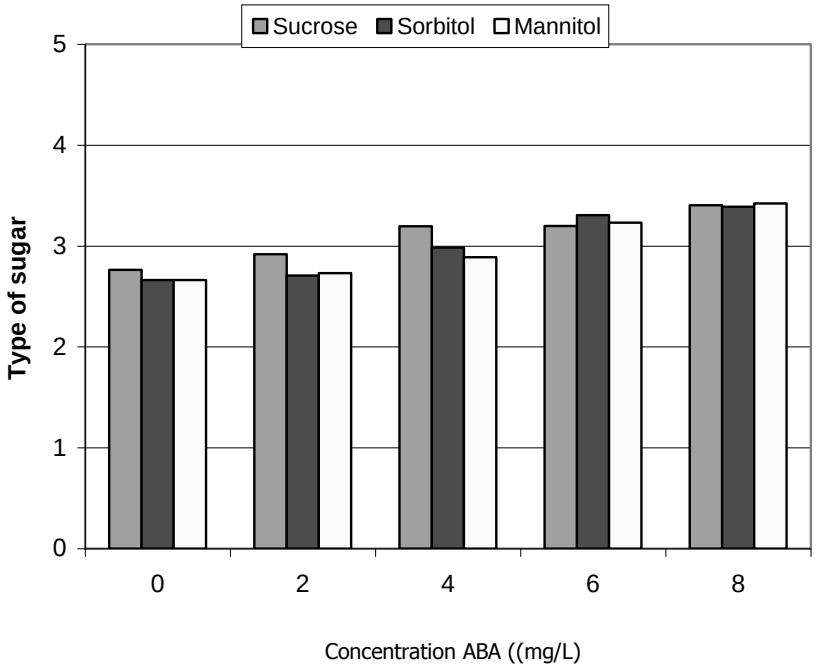
For interaction between the effect of different sugar type and different ABA concentrations added to the conservation media on the reduced sugar content of shoot tip explants, data cleared that the highest reduced sugar content was achieved from shoot tip explants conserved on medium supplemented with sucrose and 8.0 mg/l ABA.

4. Prolin amino acid content

Data in Figure (6) showed that the addition of different sugar type to conservation medium showed no significant differences on the prolin amino acid content of shoot tip explants.

Data about the effect of different ABA concentrations added to conservation medium on the proline amino acid content of shoot tip explants clearly showed that shoot tip explants conserved on ABA-free medium and medium supplemented with 2.0mg/L ABA gave the lowest significant prolin amino acid content of shoot tip explants without significant difference in between. The highest significant prolin amino acid content obtained from explants conserved on medium supplemented with 8.0 mg/L This value decreased significantly by decreasing the ABA concentration on conservation media to 6.0 and 4.0mg/L with significant difference in between.

Figure 6. Effect of different sugar and different ABA concentrations on proline amino acid content (mg\100 f.wt.) of Zaghlool cv shoot tip explants conserved at 15˚C under dark for 6and12 months.



5. Survival percentage

The shoot tip explants of each conservation treatment were transferred at the end of each conservation period (6and12 months) and recultured on normal growth medium for callus induction which consists of MS basal nutrient medium supplemented with 3.0mg/L 2iP +10.0 mg/L 2,4–D and 30.0 g/L sucrose+ 3.0g/L activated charcoal+ 5.0g/L agar and incubated under normal growth conditions (27 ± 2^{o}C under complete darkness for 24 hrs) for 4 weeks to determine the survival percentage.

Table 4. Effect of different sugar and different ABA concentrations on survival percentage of Zaghlool cv. shoot tip explants conserved at 15°C under dark for 6and12 months. (when recultured on normal growth medium and incubated under normal growth conditions for 4 weeks)

(A) Sugar 0.3M	(B) ABA mg/L	(C) Conservation Period (month)		
		6	12	(AB) Mean
Sucrose	0.0	100	100	100
	2.0	100	100	100
	4.0	100	100	100
	6.0	100	100	100
	8.0	100	100	100
Mean (A)		100	100	100 a
Sorbitol	0.0	100	100	100
	2.0	100	100	100
	4.0	100	100	100
	6.0	100	100	100
	8.0	100	100	100
Mean (A)		100	100	100 a
Mannitol	0.0	100	100	100
	2.0	100	88.88	94.44
	4.0	100	66.66	83.33
	6.0	100	66.66	83.33
	8.0	100	55.55	77.77
Mean (A)		100	75.55	87.77 b
Mean (C)		100 a	91.85 b	

(B) ABA mg/L				
0.0	2.0	4.0	6.0	8.0
100	98.14	94.44	94.44	92.59

(B) ABA mg/L	(C) Conservation Period (month)	
	6	12
0.0	100	100
2.0	100	96.29
4.0	100	88.88
6.0	100	88.88
8.0	100	85.18
L.S.D $_{0.05}$ for		
A 2.66	AB N.S	
B N.S	AC 6.84	
C 3.95	BC N.S	
	ABC N.S	

Data in Table (4) clearly showed the effect of different sugar added to conservation media on survival percentage of the shoot tip explants. conservation medium supplemented with sucrose or sorbitol seem to be the most effect on producing the highest significant survival percentages of shoot tip explants (100%) while the lowest

significant survival percentage was observed when conservation media supplemented with mannitol (87.77%).

Data regarding to the effect of different ABA conservations added to conservation media showed no significant differences on the survival percentage

Survival percentage was affected significantly by the conservation period, the highest significant survival percentage (100%)was obtained from shoot tip explants conserved for 6 months while the lowest significant survival percentage (91.85%) was obtained from shoot tip explants conserved for 12 months.

The interaction between the effect of different sugar type and conservation period on the survival percentage exhibited a significant effect. All shoot tip explants conserved for 6 months on media supplemented with sucrose, sorbitol and mannitol and shoot tip explants conserved for 12 months on media supplemented with sucrose or sorbitol can able completely to survive (100%) this survival percentage was reduced significantly to (75.55%) when shoot tip explants conserved for 12 months on medium supplemented with mannitol .

From the previous results about the conservation of shoot tip explants of date palm Zaghlool cv. data clearly showed that shoot tip explants conserved on conservation media supplemented with 0.3M from each of sucrose, sorbitol or mannitol combined with different concentrations of ABA able to initiated callus with different degree values when conserved at 5°C under dark conditions for at least 12 months while, shoot tip explants conserved on conservation media supplemented with0.3M sucrose combined with different ABA concentrations can not able to initiated callus when conserved at 15°C under dark condition for at least 12 months compared with those obtained from shoot tip explants conserved on 0.3M sorbitol media combined with different ABA concentrations which showed the highest callus initiation degree values.

In these respect Pienizak *et. al.,* (1978) mentioned that addition of ABA to the medium with 5% sorbitol initiated the growth of apple callus. Also, Shibli *et al.,(* 1999) found that increasing sucrose, (0.09 to 0.35M), sorbitol, mannitol (0.1 to 0.4M) or ABA (0.0 to 11.4M) reduced growth significantly and extended the subcultured interval to 4 months when culture were kept at room temperature. Suksa-Ard *et. al.,* (1997) Found that the effect of ABA and sucrose appeared to depend on the concentration and on the storage temperature.

From the above mentioned results it could be suggested that shoot tip explants conserved at 5°C or15°C on conservation media supplemented with high concentration of ABA (6.0or 0.8mg/L) showed the lowest callus initiation degree values compared with media supplemented with 4.0 or 2.0 mg/L ABA.

Nergi *et. al.,* (2000) found that high ABA resulted in poorer growth among medium which were used during storage period at 4°C in the dark for tow singel node shoots cultivars of apple, Watt *et. al.,* (2000) achieved slow growth storage for up to 10 months for *Eucaluptus grandis* shoot cultures by the addition of 10 mg/L ABA to the growth medium. Jarret and Gawel (1991) reported that ABA at concentrations of 0.01, 0.1 or 10.0 mg/L inhibited axillary bud and root development and subsequently plantlet growth of sweet potato.

From our results it could be clearly concluded that 86.66% of shoot tip explants conserved at 5°C for 6 months able to survived when they recultured on normal growth medium for callus initiation and incubated under normal conditions at 27 ± 2^{o}C under complete darkness for 24 hrs for 4 weeks . This percentage reduced to 45.17% by increasing the conservation period to 12 months. While, all shoot tip explants (100%) conserved at 15°C for 6 months able to survived when they recultured on normal growth medium for callus initiation and incubated under normal conditions at 27 ± 2^{o}C under complete darkness for 24 hrs for 4 weeks. This percentage reduced to 91.85% by increasing the conservation period to 12 months.

Naidu and Sreenath (1999) found that after 6 months immature zygotic embryos of *Coffea Arabia* storage on MS medium supplemented with ABA at different concentrations, the survival rate remained higher for all treatments, but it decreased gradually with prolonged duration of cultures (12,18 and 24 months), this decreased was more pronounced with low concentrations of ABA . Wang *et. al.,* (1993) reported that weak light intensity ,low temperature and chemical inhibitors (mannitol, pp333 and ABA) slowed down growth in tea germplasm storage culture and extended the preservation period.

The present data showed that 70.63% of shoot tip explants conserved at 5°C on ABA –free medium for at least 12 months able to survived when they recultured on normal growth medium for callus initiation and incubated under normal conditions at 27 ± 2^{o}C under complete darkness for 24 hrs for 4 weeks . While all shoot tip explants (100%) conserved at 15°C on ABA –free medium for at least 12 months able to survive.

In addition, increasing the concentration of ABA on conservation media from 2.0, 4.0, 6.0 to 8.0mg/L of shoot tip explants conserved at 5°C or 15°C for at least 12 months did not affect significantly the survival percentage. Suksa -Ard *et. al.,* (1997) reported that shoot growth of *Carcia papaya* was effectively inhibited and the regrowth of shoots was still satisfactory even after 12 months on medium containing 5- µM ABA.

Moriguchi *et. al.,* (1990) reported that the addition of ABA was not necessary provided the shoots of pear were stored at 5°C. Low temperature seemed to be main factor affecting the survival, on the other hand, The survival rate was significantly higher than that of the control when the cultures were stored at 10 to 15°C with 1µM ABA. However, at higher concentration of 10µM ABA at 10 or 15°C the survival rate decreased.

Watt *et. al., (*2000) found that 10mg/L ABA gave the highest survival and continued growth of *Eucalyptus grandis* shoot cultures.

Jarret and Gawel (1991) reported that ABA at 10 mg/L completely inhibited axillary shoot development but did not affect the viability of sweet potato Jewel cv. explants over a culture period of 365 days.

REFERENCES

1. A.O.A.C. 1980. Official Methods of Anal. 13th ed. Association of Official Agricultural Chemists, Washington D.C.
2. Bajaj, Y. 1986. *In vitro* preservation of genetic resources-techniques and problems. Int. Symp. Nuclear Tichniques and *In vitro* Culture for plant Improvement. IAEA/FAO Vienna PP. 43-57.
3. Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free poroline for water stress studied.Plant and Soil, 39:205-207. Cited by Zein El Din (2004) M. Sc. Thesis, plant physiology Dep., Fac. Of Agric., Ain Shams Univ., Egypt.
4. Benson, E. 1994. In plant cell culture-A practical Approach (Eds Dixon, R. and Gon zales, A.). Oxford University Press, Oxford. PP. 148-167.
5. Dubois, M., Gilles , A. K., Hamilton, J. K., Rebers and F. Smith. 1956. Colorimetric method for determination of sugar and related substance. Analyte. Chem.28:350-356. Cited by Zein El Din 2004. M. Sc. Thesis, plant physiology Dep., Fac. Of Agric., Ain Shams Univ., Egypt.
6. Engelmann, F., B. Assy-Bah, S. Bagniol, D. Dumet and N. Michaux- Ferriere. 1995. Cryopreservation of date palm, oil palm and coconut. Biotechnology in Agriculture and Forestry, Vol. 32 Cryopreservation of plant Gemplasm I (Eds. by Bajaj, Y.) Spinger- Verlag Berlin Heidelberg.
7. George, E. 1993. Plant Propagation by Tissue Culture by Bulter Tanner Ltd.
8. Jarret, R.l. and N. Gawel. 1991. Chemical and environmental growth regulation of sweet potato (*Ipomoea batatas* L. Lam) *in vitro*. Plant Cell Tiss. Org. Cult. 2: 153-160.

9. Moriguchi, I., I. Kozaki, Q. Yamaki and T. Sanad. 1990. Low temperature storage of Pear shoots *in vitro*. Bulletin of the Fruit Tree Research Station, 17: 11-18.
10. Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco cultures . Physiolo. Plant. 15:473-496.
11. Naidu, M. and H. Sreenath. 1999. *In vitro* culture of coffee zygotic embryos for germplasm preservation. Plant Cell Tiss. Org. Cult 55(3), 227-230.
12. Nergi, V., Tosti, N. and A. Standardi. 2000. Slow- growth storage of single node shoots of apple genotypes. Plan Cell Tiss. Org. Cult. 49: 81-87.
13. Ng, S. and Ng, N. 1991. Reduced – growth storge of germplasm In: *In vitro* Methods for Conservation of Plant Genetic Resources. (Eds. Dodds J.) Chapman and Hall, London. PP. 11-39.
14. Pieniazek, J., T. Holubowicz, B. K. Machnik and K. kasprzyk. 1978. Apple stem callus frost tolerance and growth modification by adding sorbitol and some growth regulators to the medium. Acta Horticultureae, 81: 91-95.
15. Pottino, B. 1981. Research on *in vitro* propagation of (*prunus Laurocensis*) cv. Ottowyken. Acta Horticultureae 300: 177-180.
16. Snedecor, G.W. and W.G. Cochran. 1972. Statistical Method 6th. The Iowa State University Press, Ames., Iowa U.S.A., 59P.
17. Shibli, R., M.: Shatnowi, M. Ajlount, A. Jaradat and Y. Adham. 1999. Slow growth *in vitro* conservation of litter Almond (*Amygdalus communis* L.) advances in Hort Sci. 13 (3): 133-134.
18. Suksa- Ard, P., I. Kataoka, Y. Fujime and S. Subhadrabandhu. 1997. Effect of temperature, growth retardants and osmotic potential on growth of Papaya shoots conserved *in vitro*. Jap. J. Trop. Agric., 41(1): 7-13.
19. Wang, L., Y. Sujuan, W. Yushu and C. Hao. 1993. Studies on indoor preservation of tea gemplasm resources by test tuble plantlets from stem cuttings. J. Tea Sci. 16 (1): 37-42.
20. Wang, Q., E. Tanne, A. Arav and R. Gatny. 2000. Cryopreservation of *in vitro*-grown shoot tips of grapevine by encapsulation- dehydration. Plant Cell Tiss. Org. Cult. 63: 41-46.

الحفظ المعملى للمنفصلات النباتية للقمم النامية لنخيل البلح تحت ظروف النمو البطىء

ميادة محمد الدوياتى[1] ، السيد إبراهيم بكر[2] ، أمينة حامد جمعه[2] ، زينب السيد زايد[1]

١. *معمل بحوث نخيل البلح – مركز البحوث الزراعية – جيزة – مصر*
٢. *قسم الفاكهة – كلية الزراعة – جامعة القاهرة – جيزة – مصر*

تضمن هذا البحث دراسة تأثير أنواع مختلفة من السكر (سكروز، سوربيتول ، مانيتول بتركيز 0.3 مول/ليتر) مع تركيزات من حمض الأبسيسيك (0.0، 2.0، 4.0، 6.0، 8.0 ملجم/ليتر) على المنفصلات النباتية للقمم النامية لنخيل البلح صنف الزغلول المحفوظ عند درجتى حرارة 5 أو 15 م° تحت الأظلام التام لمدة 6 أو 12 شهر وذلك بملاحظة التغيرات الفسيولوجية ودرجة استعادة المنفصلات النباتية قدرتها على استئناف النمو عند إعادة زراعتها تحت طروف النمو الطبيعية وذلك بهدف الحصول على أفضل الظروف للنمو البطىء أثناء الحفظ المعملى للمنفصلات النباتية للقمم النامية.

لوحظ أن جميع المنفصلات النباتية للقمم النامية والتى تم حفظها تحت درجة حرارة 5 م° لمدة 6 أشهر او تلك التى تم حفظها تحت درجة حرارة 15 م° لمدة 12 شهر على بيئة الحفظ المزودة بى السكروز أو السوربيتول مع إضافة التركيزات المختلفة لحمض الأبسيسيك كان لها القدرة على استئناف النمو.

عموما حتى الأن المعلومات قليلة عن الحفظ المعملى للقمم النامية أو الكالس الجنينى لنخيل البلح تحت ظروف النمو المنفضة للتخزين حيث يحتاج هذا الأتجاه المزيد من الدراسة لضمان إستمرارية وجود مصدر نباتى معقم خالى من التلوث من النباتات الأصلية وذلك للإنتاج المعملى التجارى.

الكلمات الدالة: نخيل البلح – الحفظ – ظروف النمو المنخفضة – السكر – حمض الأبسيسيك