10th Conf. Agric. Dev. Res., Fac. Agric., Ain Shams Univ., Cairo, Egypt, 2006
Annals Agric. Sci., Sp. Issue, 1, 149-162, 2006

GROWTH LIMITATION FOR *IN VITRO* CONSERVATION OF ORANGE GENOTYPES

[12]

Wanas, Wafaa H.[1]; I. Desouky[1]; A. El-Hammady[1] and Salwa El-Habashy,[2]

ABSTRACT

A simple system for medium term storage of *in vitro* orange germplasm cultures was developed. When using different culture vessel types and temperature of 15 or 25°C with low light intensity (20 $\mu Em^{-2} s^{-1}$) or darkness for the *in vitro* storage, both orange cvs, "Succari" and "Bedamo" stored for 36 months with survival percentage varied from 66.63% for Succari cv. in GT 25X 80 ml vessel and 77.50% for Bedamo cv. shoots in conical flasks (100ml) and both cvs incubated under low light+ 15°C. There was considerable limitation in length and number of proliferated shoots which was associated with high survival achievement. Also, a reduction in the different parameters was achieved even with the lowest concentration of Alar (0.05-0.10 mg/ litre) and ABA (10µm) added to basal MS-medium. However insignificant decrease in survival existed after 12 and 24 months storage *in vitro* at 25°C. Also high significant decrease in survival and growth parameters were associated with higher levels of Alar and ABA added to the storage medium of succari cv.

Key words: Abscisic acid, Alar, Dark, Light, Medium term, Storage, Vessel type

INTRODUCTION

Citrus species are considered the first economic fruit crops in Egypt. Succari and Ahmar Bedamo orange cvs reached 10667 feddans during 2004; while it was 20127 feddans in 1990 according to the statistics of the Ministry of Agriculture, Cairo (1990, 2004). This reflected the great reduction in the area of such cultivars. Most citrus collections are conserved in orchards, which experienced loses from biological and climatic hazards (Wanas, 1999). The germplasm diversity for any crop must be protected from any loss to ensure its availability for future plant improvement (Stuessy and Sohmer 1996).

The culture vessel and the external environment of the culture room have without doubt a tremendous influence on tissue culture system. It has been reported that type, volume and method of closure of the culture vessel had an influence on the longevity, morphology, growth and morphogenesis (Wanas, 1987; McCelland & Smith 1990; Kozai *et al* 1995; Wanas *et al* 1999 and Islam *et al* 2005).

1- Desert Research Center, El-Matariya, Cairo, Egypt.
2- Plant Protection Department, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt.

(Received September 4, 2006)
(Accepted September 13, 2006)

Also, various methods have been shown to reduce the growth rate of cultures and thus to delay the subculture frequency. Cold conditions may not be sufficient to inhibit growth so other factors in the medium such as hormones and growth retardants also have been used (**Wanas 1987 and Elobeidy 1997**).

Slow growth regimes are used as a medium–term storage option. These techniques enable subculture intervals to be extended to between 12 months and 4 years for many species, thereby reducing dramatically the laboratory space and staff time required for maintenance of cultures (**Wanas *et al* 1986 and Wanas 1987 & 1992, 1999**). Some of the factors, which could affect preservation of shoots of citrus, are being investigated in the current study.

3. MATERIALS AND METHODS

This study was achieved through the period from year 2000 until 2005 in the Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Ain Shams University, Cairo.

Establishment of *Citrus sinensis* stem node cultures

Two cultivars of orange (*Citrus sinensis*) were used through out this study namely; Acidless orange "Succari", Blood orange "Ahmar Bedamo", orange. Shoots at 10-15 cm in length were collected during the growing season (March-May) from adult trees of citrus located in the orchard of the Faculty of Agriculture, Ain Shams University. The stem nodes were surface sterilized and grown for four weeks in basal salts and vitamins of free hormones (**Murashige and Skoog, 1962**) (MS) plus 30 gl^{-1} sucrose and 7 gl^{-1} agar and maintained in the culture room for establishment.

The explants from established cultures were transferred to jars (85 x 50 mm) filled with 35 ml MS salts and vitamins at full strength plus (as mgl^{-1}) (0.2) benzyl amino purine (BAP) and (0.5) indole-3-butyric acid (IBA), 30 gl^{-1} sucrose, and kept in the culture room as a stock for the storage experiments.

2.1. *In vitro* storage under minimal environmental conditions

Two concepts in this respect were studied on both Acidless orange "Succari" and Blood orange "Ahmar Bedamo" as follow:

2.1.1. Effect of culture vessel types and different environmental conditions on storage of shoot culture of orange

In this experiment, the shoot tips (10mm) from orange cvs were cultured in the different culture vessels as follow:

1. Glass test tubes (T.T) (15 X 150mm) capped with aluminium foil filled with 13 ml of the media.
2. Glass tubes (G.T) (25 x 80) and (25 x 150mm) which is capped with screw caps or Bellco Kaputes respectively and filled with 20 ml of the media.
3. Glass jars (50 x 85 mm) (GJ 200 ml) and (70 x 120 mm) (350ml jar) capped with polypropylene lids (caps), filled with 60 ml of media.
4. Conical flask (100 ml) (C.F) capped with aluminium foil, filled with 60 ml of the media. All previous treatments stored at normal conditions (25°C±2).

The cultures were incubated either at 16 h. day photoperiod and low light intensity of 20 $\mu Em^{-2} s^{-1}$ at 25°C and 15°C or in dark under 15°C, the data were collected every 6 months.

2.1.2. Effect of using growth retardants in the medium on *in vitro* storage

In this experiment ABA at different concentrations (0.0, 10, 100, 500, 1000 µM and Alar: 0.0, 0.05, 0.1, 0.5, 1 mg/l were added to MS salts and vitamins.

Test tubes 15 x 150 mm were used in this concept for 12 months. After that, the shoots were transferred to glass tubes 25 x 150 mm. The cultures were incubated at 25°C and normal light intensity of 30 $\mu Em.^{-2} s^{-1}$.

Each treatment consisted of six replicates, three shoots for each replicate in a completely randomized design. Duncan's multiple range test was employed for means comparisons according to (**Sendecor and Cochran, 1982**).

4. RESULTS AND DISCUSSION

4.1. *In vitro* storage under minimal environmental conditions

4.1.1. Effect of culture vessel types and different environmental conditions on survival percentage of Succari orange during *in vitro* storage

Tables from 1-3 clarify the data on storage of Succari shoots for different periods (12, 24 and 36 months) under different environmental conditions including (light or darkness) and different temperatures (15 or 25°C). **Table (1)** cleared that after storage for 12 months, all tested vessels gave high survival percentages without significance among them. Concerning the storage conditions, there were insignificant differences among them. Regarding the interactions, all environmental treatments with different types of vessels recorded 94.4-100 survival percentages with insignificant differences among them.

After storage for 24 months, all vessel types gave high survival % without significance among them. Regarding the storage conditions, the storage at 15°C either at light or darkness recorded the highest significant survival % compared with the storage at light +25°C which gave the lowest significant survival% (59.54). Regarding the interactions, TT15x150 mm and GJ 200 ml at light+ 15°C or dark showed the highest survival %, however, the differences among treatments were insignificant.

After 36 months of storage, the same trend was observed for the specific effect of both vessels types or the storage environmental conditions. Regarding the interactions, the storage of Succari shoots under light +15°C in GT 25x80 mm gave the highest survival %(66.63) while, the lowest survival % was obtained by GJ 200 ml at light +25°C (33.30). Storage at 15°C either in light or darkness gave the best survival % after 24 months when using TT or GT. While after 36 months the survival% decreased to 66% approximately under 15°C+light using GT 25x80mm. So, TT and GT are considered the best vessels for storage especially at low temperature.

Table 1. Effect of culture vessel type and different environmental conditions on survival percentage of Succari orange shoots during *in vitro* storage.

Vessel types	Light+25°C	Light+15°C	Dark+ 15 °C	Mean
	After 12 months			
TT 15x150mm	94.43a	94.33a	100.00a	96.26A
GT25x80mm	94.43a	100.00a	100.00a	98.14A
GJ200ml	100.00a	94.43a	94.43a	96.26A
CF 100ml	94.43a	100.0a	100.00a	98.14A
Mean	95.82A`	97.19A`	98.58A`	
	After 24 months			
TT 15x150mm	61.07ab	88.67a	88.67a	79.47A
GT25x80mm	77.50a	83.00a	77.33a	79.28A
GJ 200ml	49.65b	88.67a	83.00a	73.77A
CF 100ml	49.95b	83.22a	77.55a	70.24A
Mean	59.54B`	85.89A`	81.64A`	
	After 36 months			
TT 15x150mm	38.85bc	49.95a-c	61.07ab	49.96A
GT25x80mm	38.85bc	66.63a	49.95a-c	51.81A
GJ 200ml	33.30c	49.95a-c	49.95a-c	44.40A
CF 100ml	38.85bc	55.50a-c	44.30a-c	46.22A
Mean	37.46B`	55.51A`	51.32A`	

Means followed by the same letter(s) are not significantly different from each other at 5% level.

In this concern, **Wanas (1987); Kozai *et al* (1995) and Wanas *et al* (1999)** discussed the influence of culture vessel on the longevity and growth rate of stored cultures. Also, **Islam *et al* (2005)** reported that the culture tube gave the lowest weight loss from the media and fresh weight for mint cultures.

4.1.2. Effect of culture vessel types and different environmental conditions on number of new proliferated shoots of Succari orange shoots during *in vitro* storage

Table (2) showed that after 12 months, the large vessels (GJ 200ml, CF 100ml) relatively produced high significant mean number of new shoots

Table 2. Effect of culture vessel type and different environmental conditions on number of new proliferated shoots of *Succari orange* shoots during *in vitro* storage.

Vessel types	Light+25°C	Light+15°C	Dark+ 15 °C	Mean
	After 12 months			
TT 15x150mm	1.05c	0.35d	0.30d	0.57B
GT25x80mm	1.33c	0.35d	0.30d	0.66B
GJ200ml	2.83a	0.35d	0.30d	1.16A
CF 100ml	2.50b	0.49d	0.33d	1.11A
Mean	1.93A`	0.38B`	0.31B`	
	After 24 months			
TT 15x150mm	1.97c	0.59d	0.30d	0.95B
GT25x80mm	2.66b	0.55d	0.30d	1.17B
GJ200ml	4.17a	0.35d	0.35d	1.62A
CF 100ml	4.42a	0.44d	0.44d	1.76A
Mean	3.31A`	0.48B`	0.35B`	
	After 36 months			
TT 15x150mm	1.97c	0.60d	0.56d	1.02C
GT25x80mm	2.80b	0.60d	0.60d	1.33B
GJ 200ml	4.17a	0.73d	0.45d	1.78A
CF 100ml	4.17a	0.56d	0.56d	1.76A
Mean	3.27A`	0.62B`	0.53B`	

Means followed by the same letter(s) are not significantly different from each other at 5% level.

compared with small vessels (TT 15 x 15, GT 25 x 8 mm). The differences among them were significant. Also, the storage at low light + 25°C gave the highest significant mean (1.93) compared with other treatments which produced relatively a little number of shoots. The interactions cleared that the highest significant number was recorded in GJ 200 ml when stored under light + 25°C (2.83). Also, the storage at 15°C with light or dark gave parallel values with insignificant differences among them. The same trend was recorded after 24 months as CF, also, recorded high number of shoots.

Also, the interactions were similar except that CF vessel under normal environmental conditions recorded high significant number of shoots as GJ 200 ml. After 36 months, both GJ 200ml and CF gave the highest significant mean, whereas the lowest significant mean was in TT (1.02). Regarding the treatments, low light + 25°C gave the highest significant mean (3.27) compared with storage in light or dark +15 °C. The interactions cleared that CF and GJ 200ml under low light + 25°C gave the same high significant values. The storage at 15°C with light or dark recorded similar lower values.

4.1.3. Effect of culture vessel types and different environmental conditions on length of new proliferated shoots (cm) of Succari orange shoots during *in vitro* storage

Table (3) illustrates the effect of vessel types and storage at different conditions on the length of new proliferated shoots. After 12 months, the large vessels (GJ 200ml, CF 100ml) gave relatively higher significant mean length of new shoots (1.31 and 1.19) compared with small vessels TT and GT .The differences among them were significant. Also, the storage at low light + 25°C gave highest significant mean (2.10) compared with other treatments which gave lower mean length of shoots. The interactions cleared that highest significant length was recorded in GJ 200 ml and CF when stored low light + 25°C (2.66 and 2.66). Also, the storage at 15°C with light or dark gave approximately parallel values with insignificant differences among them.

After the storage for 24 months, CF 100ml recorded the highest mean while, TT gave the lowest significant mean (0.87). The effect of the different environmental conditions showed similar trend in interaction as shownafter 12 months storage. After 36 months storage, the data showed typical trend as after 12 and 24 months with approximately parallel means with insignificant differences among them.

Table 3. Effect of culture vessel type and different environmental conditions on length of new proliferated shoots of Succari orange shoots during *in vitro* storage

Vessel types	Light+25°C	Light+15°C	Dark+ 15 °C	Mean
	After 12 months			
TT 15x150mm	1.51b	0.63c	0.58c	0.91B
GT25x80mm	1.55b	0.52c	0.48c	0.85B
GJ200ml	2.66a	0.40c	0.52c	1.19A
CF 100ml	2.66a	0.55c	0.72c	1.31A
Mean	2.10A`	0.52B`	0.57B`	
	After 24 months			
TT 15x150mm	1.33c	0.71d	0.56d	0.87D
GT25x80mm	2.81b	0.57d	0.66d	1.34C
GJ200ml	3.92a	0.48d	0.62d	1.67B
CF 100ml	4.25a	0.88d	0.72d	1.95A
Mean	3.07A`	0.66B`	0.64B`	
	After 36 months			
TT 15x150mm	1.33d	0.77e	0.96de	1.02D
GT25x80mm	2.66c	0.72e	0.90de	1.43C
GJ200ml	3.66b	0.73e	0.83de	1.74B
CF 100ml	4.25a	0.87de	0.96de	2.03A
Mean	2.97A`	0.77B`	0.92B`	

Means followed by the same letter(s) are not significantly different from each other at 5% level.

4.1.4. Effect of culture vessel types and different environmental conditions on survival percentage of Ahmar Bedamo orange shoots during *in vitro* storage

Table (4) cleared that after storage for 12, 24 and 36 months, all vessels gave high survival % without significant differences. Concerning the environmental conditions, there were insignificant differences among them after 12, 24 or 36 months. Regarding the interactions, all treatments recorded insignificant differences among them except CF under 15°C+light which gave the lowest significant survival% (83.32) after 12 months, while after storage for 24 and 36 months the interactions between the two studied variables were insignificant.

4.1.5. Effect of culture vessel types and different environmental conditions on number of new proliferated shoots of Ahmar Bedamo orange shoots during *in vitro* storage

As shown in Table (5), it is clear that the specific effect of vessel types was similar after 12,24 and 36 months storage as GJ 200 ml followed by CF 100 ml were the highest in mean number of proliferated shoots with insignificant difference between them. At the same time, light and temperatures condition cleared that low light + 25°C recorded the highest significant mean after 12, 24 and 36 months in storage.

Table 4. Effect of culture vessel type and different environmental conditions on survival percentage of Ahmar Bedamo orange shoots during *in vitro* storage

Vessel types	Light+25°C	Light+15°C	Dark+ 15 °C	Mean
	After 12 months			
TT 15x150mm	94.43ab	100.00a	100.00a	98.14A
GT25x80mm	100.00a	100.00a	100.00a	100.00A
GJ 200ml GJ	100.00a	94.43ab	88.87ab	94.43A
CF 100ml	100.00a	83.32b	94.43ab	92.58A
Mean	98.61A`	94.44A`	95.82A`	
	After 24months			
TT 15x150mm	77.73a	88.67a	88.67a	85.02A
GT25x80mm	71.98a	83.00a	77.33a	77.44A
GJ 200ml	88.67a	88.67a	83.00a	86.78A
CF 100ml	88.67a	77.50a	83.17a	83.11A
Mean	81.76A`	84.46A`	83.04A`	
	After 36 months			
TT 15x150mm	38.85a	60.67a	60.67a	53.39A
GT25x80mm	49.98a	44.00a	60.67a	51.55A
GJ 200ml	66.38a	72.00a	60.77a	66.38A
CF 100ml	60.87a	77.50a	49.92a	62.76A
Mean	54.02A`	63.54A`	58.00A`	

Means followed by the same letter(s) are not significantly different from each other at 5% level.

Table 5. Effect of culture vessel type and different environmental conditions on number of new proliferated shoots of Ahmar Bedamo orange shoots during *in vitro* storage.

Vessel types	Light+25°C	Light+15°C	Dark+ 15 °C	Mean
	After 12 months			
TT 15x150mm	1.15b	0.42c	0.40c	0.66B
GT25x80mm	1.22b	0.40c	0.40c	.69B
GJ 200ml GJ	2.38a	0.67c	0.45c	1.17A
CF 100ml	2.22a	0.42c	0.45c	1.03A
Mean	1.74A'	0.47B'	0.44B'	
	After24 months			
TT 15x150mm	1.11bc	0.83cd	0.76de	0.92B
GT25x80mm	1.25b	0.63de	0.55de	0.81B
GJ 200ml	2.61a	0.62de	0.45e	1.23A
CF 100ml	2.36a	0.72de	0.45e	1.18A
Mean	1.83A'	0.70B'	0.55B'	
	After 36 months			
TT 15x150mm	0.77b	0.66b	0.68b	0.71B
GT25x80mm	0.75b	0.53b	0.45b	0.57B
GJ 200ml	2.61a	0.52b	0.45b	1.19A
CF 100ml	2.08a	0.72b	0.40b	1.06A
Mean	1.55A'	0.61B'	0.49B'	

Means followed by the same letter(s) are not significantly different from each other at 5% level.

Also, the same trend was true after 12,24 and 36 months for the interactions where GJ200 ml with light +25°C achieved the highest significant number of proliferated shoots and other regimes showed insignificant differences.

4.1.6. Effect of culture vessel types and different environmental conditions on length (cm) of new proliferated shoots of Ahmar Bedamo orange shoots during *in vitro* storage

After 12 months (Table 6) it is clear that the specific effect of vessel types was highest in GJ 200 ml in mean length of proliferated shoots. At the same time light and temperatures condition cleared that light+ 25°C(control) recorded the highest significant mean after 12 ,24 and 36 months in storage. Also, the same trend was true after 12,24 and 36 months for the interactions where GJ200 ml with light +25°C achieved the highest significant length of proliferated shoots and other regimes showed insignificant differences.

Cultures at any of the reduced temperature either with light or darkness depressed the growth rate and enhanced survival % compared with the light + 25°C regimes. The results of the present experiment indicated that it is possible to extend the interval between subcultures for at least 24 months with nearly 90%

Table 6. Effect of culture vessel type and different environmental conditions on length of new proliferated shoots of Ahmar Bedamo orange shoots during *in vitro* storage.

Vessel types	Light+25°C	Light+15°C	Dark+ 15 °C	Mean
	After 12 months			
TT 15x150mm	1.16d	0.66ef	0.66ef	0.84C
GT25x80mm	1.55c	0.62ef	0.58ef	0.92C
GJ200ml	3.17a	0.66ef	0.60ef	1.48A
CF 100ml	2.69b	0.76e	0.40f	1.28B
Mean	2.14A`	0.67B`	0.56B`	
	After 24 months			
TT 15x150mm	1.30c	0.83d	0.66d	0.93B
GT25x80mm	1.58c	0.56d	0.66d	0.94B
GJ200ml	3.33a	0.66d	0.70d	1.56A
CF 100ml	2.61bl	0.70d	0.90d	1.40A
Mean	2.21A`	0.69B`	0.73B`	
	After 36 months			
TT 15x150mm	0.88c	0.66c	0.58c	0.71B
GT25x80mm	0.92c	0.46c	0.56c	0.65B
GJ 200ml	3.33a	0.56c	0.70c	1.53A
CF 100ml	2.33b	0.70c	0.76c	1.27A
Mean	1.86A`	0.59B`	0.65B`	

Means followed by the same letter(s) are not significantly different from each other at 5% level.

survival under light or darkness at 15 or 25°C. The cultures showed reduced growth (as presented by low number of shoots and lower length) and did not require transfer or any other renewed procedure to extend the overall storage term. However survival percentage was higher only at 15°C + light after 36 months, for both cvs when using conical flasks.

That means that the type of culture vessel can play an important role, and has a strong effect on growth and survival percentage during the storage period. **Monette (1986)** found that the small size of the kiwi culture vessel led to a decrease in length and number of shoots. **Islam *et al* (2005)** reported that beneficial effects of larger air volumes or larger vessels interpreted by causing a better composition of the gaseous components as carbon dioxide, oxygen and ethylene, mainly by retarding accumulation of unfavourable gases.

The authors suggested that the vessel type affects the internal CO_2, ethylene and other volatiles in the airspace within the container, which in turn had a stronger influence on gas exchange. Moreover, the vessel shape affects the amount of medium exposed to the atmosphere inside the vessel, which can affect the rate of moisture loss and diffusion of gaseous compounds. The vessel closures are important due to the control of the release of gaseous compounds built up during plantlet growth and the control of moisture loss (**Kavanagh *et al* 1991**).

4.2. Minimal growth medium with growth retardants

4.2.1. Influence of Alar (B9) on the survival percentage and growth of Succari orange shoots *in vitro* at 25°C.

After storage for 12 months (Table 7), high significant survival% (85.92) was compared with 65.0% survival after storage for 24 months. Concerning the effect of different concentrations of Alar, there were insignificant differences between zero, 0.05 and 0.10 mgl^{-1} of Alar, with survival % (83.31, 86.0 and 83.17), respectively followed by 0.5 mgl^{-1} which recorded (77.50). While the lowest significant survival was recorded with 1.0 mgl^{-1} (55.17). The interactions revealed that the storage for 12 months with 0.05,0.10 and 0.5 mgl^{-1} Alar recorded high survival% (100, 88.67 and 88.67) with insignificant differences. The lowest survival % obtained after storage for 24 months with Alar at 1.0 mgl^{-1} .

The storage for 12 and 24 months recorded insignificant difference between the numbers of new shoots (Table 7). Regarding the role of treatments of Alar, 0.10 mgl^{-1} gave the highest significant mean (2.33) while 0.05, 0.5 or 1.0 mgl^{-1} gave lower significant values than zero level. The interactions revealed that storage for 12 months with Alar at 0.10 mgl^{-1} recorded the highest significant number of new proliferated shoots (2.52) followed by the (2.14) after storage for 24 months. Other concentrations at 0.50 or 1.0 mgl^{-1} recorded the lowest mean after 12 and 24 months.

The mean length of new shoots recorded insignificant differences between the two storage periods (12, 24 months). Regarding the role of Alar, (0.10, 0.5, 1.00 mgl^{-1}) showed significant decrease in mean length compared with the control. While 0.05 was insignificantly lower than control. The interactions revealed that Alar at 0.05 mgl^{-1} with either storage for 12 or 24 months gave the same mean (1.50) with significant differences with other treatments except the control.

Table 7. Effect of Alar (B9) on the survival % and growth of Succari orange shoots stored *in vitro* at 25°C.

Survival%

Alar as (mgl^{-1})	Storage period		
	12 months	24 months	Mean
0	94.83a	72.18ab	83.31 A
0.05	100.0a	72.00ab	86.00A
0.10	88.67a	77.67ab	83.17A
0.50	88.67a	66.33ab	77.50AB
1.00	66.33ab	44.00b	55.17B
Mean	85.92A`	65.00B`	
	Number of new proliferated shoots		
0	1.97a	3.00a	2.48A
0.05	1.74b	1.70b	1.73B
0.10	2.52a	2.14ab	2.33A
0.50	1.00c	0.93c	0.96C
1.00	0.72c	0.08c	0.76C
Mean	1.59A`	1.57A`	
	Average of new shoots		
0	2.16a	3.08a	2.62A
0.05	1.50a	1.50b	1.50A
0.10	0.98b	0.92b	0.95B
0.50	0.90b	0.80b	0.85B
1.00	0.63b	0.75b	0.69B
Mean	1.23A`	1.41A`	

Means followed by the same letter(s) are not significantly different from each other at 5% level

4.2.2. Influence of Abscisic acid (ABA) on the survival and growth of Succari orange shoot cultures stored *in vitro* at 25°C

Survival of Succari orange shoots (Table 8), after storage for 12 months, gave highest significant survival% (94.36) compared with storage for 24 months (53.20). Regarding the effect of ABA, all concentrations of ABA gave insignificant differences among them except ABA at 1000µM which showed the lowest mean significant survival %(63.58). The interactions between ABA conc. and storage period revealed that the storage for 12 months with 10,100 and 500µM ABA recorded the highest survival% (94.33-100). The lowest survival % appeared after storage for 24 months with ABA at 1000 µM (38.50).

The storage for 12 and 24 months recorded insignificant difference in numbers of new shoots. Regarding the role of treatments of ABA, significant differences were recorded between (0 and 10, 100) and also between (10,100 and 500,1000). The interactions revealed insignificant differences between treatments in the effect on number of proliferated shoots during storage except for the significant reduction at 500 and 100 µM after 24 months storage (Table 8).

The storage for 12 and 24 months, recorded insignificant difference in the lengths of the new shoots. Regarding the role of treatments of ABA, 0 and 10µM levels gave the highest significant means (2.98 and 2.08), respectively compared with the other concentrations, which recorded insignificant differences among them. The interactions revealed that ABA at 100, 500 and 1000µM decreased the shoot length either after storage for 12 or 24 months started to decrease the length of the new shoots. Also other concentrations recorded the lowest means with insignificant differences among them (Table 8).

Table 8. Effect of Abscisic acid (ABA) on the survival and growth percentage of Succari orange shoots stored *in vitro* at 25 °C.

ABA as (µM)	Storage period 12 months	Storage period 24 months	Mean
	Survival%		
0	88.80b	72.18b	80.49A
10	100.0a	66.33bc	83. 17A
50	94.33a	55.00c	74.67A
100	100.0a	44.00c	72.00A
1000	88.67b	38.50c	63.58B
Mean	94.36A`	53.20B`	
	Average number of new proliferated shoots		
0	2.61a	3.00a	2.85A
10	1.50a	1.17a	1.33B
100	1.25a	1.00a	1.12B
500	1.00a	0.75b	0.87C
1000	1.08a	0.75b	0.92BC
Mean	1.49A`	1.33A`	
	Average length of new shoots		
0	2.16a	3.80a	2.98A`
10	2.33a	1.83a	2.08A
100	0.92b	0.75b	0.83B
500	0.58b	0.41b	0.50B
1000	0.58b	0.33b	0.45B
Mean	1.31A`	1.42A`	

Means followed by the same letter(s) are not significantly different from each other at 5% level

It is worthy to mention that growth retardants or growth inhibitors could limit growth during storage of shoots with or without the establishment of longevity of shoot cultures. The use of growth inhibitors has been attempted by (**Roca *et al* 1982 and Wanas, 1987**) for cassava and pear germplasm. However, the authors indicated that ABA was detrimental to some varieties.

Elobeidy (1997) added ABA at 1 µM to shoots of "Shobra' pear storage media at 4°C. The percentage of survival was reduced in the presence of ABA in the culture media. ABA has a negative effect on the efficiency of the storage of cultures at low temperatures.

Saftner and Wyse (1984) reported that the rapid effect of ABA on sucrose transport indicates a relatively close association between the physiological activities of ABA and the operation of active transport system. Thus ABA affected the accumulation of sucrose and other assimilates in certain tissue.

REFERENCES

Elobeidy, A.A. (1997). *In vitro* conservation of "Shobra"pear at low temperature. ***Egypt. J. Plant Breed.1: 103-108.***

Islam, T.M.; D.P. Dembele and E.R.J. Keller (2005). Influence of explant, temperature and different culture vessels on *in vitro* culture for germplasm maintenance of four mint accessions. ***Plant Cell, Tissue & Organ Culture, 81: 123-130.***

Kavanagh, K.; A.P. Drew and C. Maynard (1991). The effect of culture vessel on micropropagtion. ***Biotechnology Agric. and Forestry. (17) High Tech and Micropropagation II pp. 202-211*** (ed. Bajaj Y.P.S.) Springer–Verlag Berlin.

Kozai, T.; B.R. Jeong; C. Kubota and Y. Murai (1995). Effect of volume and initial strength of medium on the growth, photosynthesis and ion uptake of potato (*Solanum tuberosum* L.) plantlet *in vitro*. ***Journal of the Japanese Soc. of Hort. Sci., 64(1): 63-71.***

McClelland, M.T. and M.A.L. Smith (1990). Vessel type, closure, and explant orientation influence *in vitro* performance of five woody species. ***HortScience, 25(7):797-800.***

Monette, P.L. (1986). Cold storage of kiwi fruit shoot tips *in vitro*. ***HortScince 21(5):1203-1205.***

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

Roca, W.M.; J. Rodigues; J. Beitran; J. Roa and G. Mafla (1982). Tissue culture for the conservation and international exchange of germplasm in: ***Plant Tissue Culture, pp.711-771*** (ed. Fujiwara, A.). Japan Assoc. Plant Tissue Cult., Tokyo.

Saftner, R.A. and R.E. Wyse (1984). Effect of plant hormones on sucrose uptake by sugar beetroot tissue discs. ***Plant Physiol., 74: 951-955.***

Snedocor, G.W. and W.G. Cochran (1982). ***Statistical Methods. 507 pp. 7th Ed.*** Iowa State University Press Ames ,Iowa, USA.

Stuessy, T.F. and S.H. Sohmer. (1996). Sampling the green world. In: ***Innovative Concepts of Collection, Preservation and Storage of Plant Diversity, 289 pp.*** Colmbia University Press, New York. USA.

Wanas, Wafaa, H. (1987). ***Genetic Conservation of Pyrus spp. Using Tissue Culture Techniques. pp. 116-134.*** Ph.D. Thesis, Brimingham University, England, U.K.

Wanas, Wafaa, H. (1992). *In vitro* storage of proliferated apple rootstock shoot- tip cultures. *Annals Agri. Sci., Ain Shams Univ. Cairo, 37(2): 501-510.*

Wanas, Wafaa, H. (1999). The application of *in vitro* technique for the genetic conservation of some *Citrus* spp. *Annals Agric. Sci., Ain Shams Univ. Cairo, 44(2): 653-672.*

Wanas, Wafaa, H.; J.A. Callow and L.A. Withers (1986). Growth limitation for the conservation of pear genotypes. In: *Plant Tissue Culture and its Agricultural Application. pp. 285-290.* (Eds. Withers, L.A. and P.G. Alderson), Cambridge University Press, London.

Wanas, Wafaa, H.; A.M. El-Hammady; M.R. Tadrous and S. El-Habashy (1999). Microprpagation of citrus rootstocks: Effect of vessel type and the physical nature of the medium on multiplication and rooting of the shoot tip cultures. *The VI th National Conference on Environmental Studies & Research, 533-545.*

المؤتمر العاشر لبحوث التنمية الزراعية، كلية الزراعة، جامعة عين شمس، القاهرة، مصر، ٢٠٠٦

مجلد خاص، حوليات العلوم الزراعية، عدد خاص، ١: ١٤٩-١٦٢، ٢٠٠٦

تقليل النمو من أجل تخزين التراكيب الوراثية لصنفين من البرتقال في المعمل

[١٢]

وفاء حسانين ونس[١]- إبراهيم دسوقي[١]- عبد العظيم الحمادى[١]- سلوى الحبشى[٢]

١- كلية الزراعة- قسم البساتين- شبرا الخيمة- القاهرة- مصر

٢- قسم التربية- مركز بحوث البساتين-الجيزة- مصر

في هذا البحث تم وضع نظام مبسط لتخزين مزارع الأنسجة التي تمثل بعض الأصول الوراثية للبرتقال . وقد تم استخدام أنواع مختلفة من أوعية الزراعة والتحضين على درجات حرارة ١٥، ٢٥ °م إلى جانب إما كثافة ضوئية منخفضة (٢٠ ميكروإنستين/ متر مربع/ ثانية) أو إظلام وتم تخزين كلا من صنفي السكري وأبو دمه لمدة ٣٦ شهر بدون نقل Subculture وتراوحت نسبة البقاء ٦٣ر٦٦% لصنف السكري المخزن فى أنابيب زجاجية ٢٥ X ٨٠ ملي و ٧٧,٥٠% لصنف أبو دمه المخزن فى دوارق مخروطية حجم ١٠٠ ملي وكلاهما تم تحضينه في ظروف إضاءة منخفضة + ٥١°م. وأدت هذه الطريقة إلى تحديد معنوي في أطوال وعدد الفريعات المتكاثرة أثناء التخزين والذي نتج عنه تحقيق نسبة بقاء عالية.

أيضا كان هناك نقص واضح فى عدد البراعم المتكاثرة وأطوالها حتى مع أقل التركيزات من الآلار (٠,٥، ١,٠ ملجم/ لتر) وحمض أبي سيسك (١٠ ميكرومولر)

المضافين لبيئة التخزين الغير محتوية على الهرمونات (أملاح بيئة موراشيج وسكوج فقط) ولو أن ذلك أدى أيضا إلى تناقص غير معنوي في نسبة البقاء بعد ١٢ ، ٢٤ شهر من التخزين في المعمل على درجة ٢٥°م.

أيضا أدت التركيزات العالية من الآلار وحمض الابيسيك المضافة لبيئة التخزين إلى تحديد كبير للنمو صاحبه انخفاض معنوي في نسبة البقاء للمزارع المخزنة من البرتقال السكري.

تحكيم: ا.د محمد أبورواش علي بدر
ا.د محمد كمال بحر