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Some Factors Affecting The *In Vitro* Storage of "Succari" Orange Cultures at Normal Growth Conditions

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THIS WORK explores the possibility of using *in vitro* techniques to store the shoots of "Succari" orange (*Citrus sinensis* L). In this study both the media composition and the vessel type were investigated. The shoot cultures were stored up to 36 month (without subculturing) in normal conditions; (16 hr. light and 8 hr. dark, the light intensity was about 2500 Lux at explants level at 25±2°C). Media consisted of, MS at full strength without growth regulators (MSF); MS at half strength without growth regulators (½MSF) and MS at full strength plus (mgl^{-1}) 0.4 IBA, 1.0BA, 126 phloroglucinol. The same tested media were used in different culture vessels. The vessel types were test tubes15x150mm (TT.), glass tubes 25x 80 mm 25 x 150 mm (GT.), glass jars 200ml and 350ml (GJ.) and conical flask 100ml (CF.). Data were recorded every 12 months storage period. The highest survival percentages were recorded in GT.25 x 150mm & CF. filled with MSF and in TT 15 x 150 mm & 25 x 80 mm which were filled with ½MSF, while the other vessels (GJ 200ml and 350ml) had recorded the lowest survival percentage during the storage period (36months) especially with MSH. Shoots stored in a small container for 36 months (such as TT, GT 25x 80, 25x150 mm and CF.) showed good healthy appearance. Regarding the length of the original shoots, the highest length was recorded with CF. filled with MSF and the lowest length was recorded when GJ. 350ml filled with MSH was used. The highest number of leaves was found when the GT.25x 80 mm and 25x150 mm filled with MSH or the GJ.200 ml filled with MSF were used. The lowest number was recorded with GJ. 350ml plus MSH. The highest number of new shoots was recorded with CF. filled with MSF, however the lowest number was in GJ. 350 ml with the same medium. Regarding the length of new shoots, the highest length was in CF. with MSF and GT.25x 80mm with MSH while the lowest one was recorded with GT 25x150mm with ½ MSF. In recovery experiment, the growth rate was slow in the first subculture after storage, then it was increased gradually during the second and third subcultures. It was evident that shoots cultured in large volume vessels during storage was more efficient in proliferating shoots during recovery compared to shoots cultured in small storage one.

Keywords: Genetic conservation, *In vitro*, Vessel types, Citrus, Succari cv.

Citrus species are considered the first economic fruit crop in Egypt. Its cultivated area occupies about "352768" feddans. Succari orange reached about 11065

feddans during 2003; while in 1990 it was 20127feddans according to the statistics of the Ministry of Agriculture, Cairo (1990,2003). This reflected the great reduction in the area of such cultivar. Conservation of citrus genetic resources is subjected to the limitations experienced with most woody perennials. Most citrus collections are conserved in field plantings which experienced losses from biological and climatic hazards (Withers & King 1980 and 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 germplasm of citrus should cover primitive, old and current cultivars (Rathore *et al.*, 1993). Alternatively, *in vitro* storage of shoot cultures provides a source of explants protected from contamination and available for any new micropropagtion. It is also, an accepted system for storage with high degree of genetic stability (Kartha *et al.* 1981, Wanas *et al.* 1986, Wanas 1992 & 1999 and Marin & Duran Vila, 1991). Attempts to maintain some citrus species for short term based on the periodic recovery of rooted plantlets. The use of enclosed containers in tissue culture system interrupts the flow of secondary products away from the developing plant. This can have a variety of consequences, both harmful and beneficial, depending on the type of the type of cultured tissue, the species and the culture vessel types (Mullin & Schlegal, 1976, Roca *et al.* 1982, Wanas, 1987, McClelland & Smith, 1990 and Kavanagh *et al.* 1991). Preservation protocols were developed for approximately 40 tropical plant species (Engelmann, 1991). Accordingly, the aim of this study was the determination of the effect of culture vessel and closure types and the type of enclosed medium on the *in vitro* storage of orange(*Citrus sinensis*) Succari cv. shoot tip cultures.

Material and Methods

This investigation was carried out in the Tissue Culture Laboratory, Horticulture Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt, during the period from 2000-2004. The shoot cultures of " Succari" were initiated *in vitro* using stem pieces with length from 0.5-1.0 cm, collected from adult trees located in the orchard of the Faculty of Agriculture, Ain Shams University, during the growing season. The stem pieces were disinfected with 10% Clorox (v/v) for 15 min before rinsing for 3 times with sterile distilled water. The cultures were maintained at 25±2°c with 16 hr photoperiod and $40\mu E^{s-1}$ light intensity as a normal growing conditions until being used as a mother stock for storage experiment.

Storage experiment

Influence of culture vessel types and media composition on the" Succari" orange culture during in vitro storage

The cultures initiated and multiplied for three subcultures in the same medium mentioned later before being used as a source for this experiment. The basic salts and vitamins of Murashige and Skoog, (1962) (MS) medium were prepared as follow:

A- MS at full strength plus $30gl^{-1}$ sucrose without growth regulators (MSF).

B- MS at half strength plus $15gl^{-1}$ sucrose without growth regulators (½MSF).

C- MS at full strength supplemented with (mgl^{-1})0.4 IBA, 1.0 BA, 126 phloroglucinol and $30gl^{-1}$ sucrose (MSH), this medium was used as a control.

All types of media were supplemented with $7gl^{-1}$ agar; the pH of the media was adjusted to 5.8 before autoclaving. Autoclaving was done at 100 k .pa (15 P.S I) and 121c° for 20 min. All the cultures were stored under normal growth conditions. The culture vessels used in this experiment were as follow:

1- Glass test tubes (15x150 mm) capped with aluminum foil filled with 13 ml of the different types of media (TT.)

2- Glass tubes (25x80 mm) and (25x150 mm), capped with screw caps or Bellco Kaputes, respectively and filled with 20 ml of the different types of media (GT).

3- Glass jars (50x85 mm) (200 ml GJ.) and (70 x 120 mm) (350 ml GJ.) capped with polypropylene lids (caps), (both jars 200 and 350 ml) filled with 60ml of the different types of media (GJ).

4- Conical flask (100 ml) capped with aluminum foil, filled with 60ml of the different types of media (CF).

Each treatment consisted of six replicates; each replicate was represented by 3 tubes (one shoot per tube) in TT., GT.25x80 mm and 25x150 mm and three shoots per 200ml GJ., 350ml GJ. and CF. in a completely randomized design . The data were recorded every 12 months during the storage period.

The criteria of storage efficiency were survival percentage (Number of survived shoots /Total number of stored shoots) x100, length of original shoots, total number of leaves, length and number of proliferated shoots. During periods of storage, the level of media and the morphology of shoots were noticed every 6 months intervals.

The data were statistically analyzed according to M –STAT program as a factorial experiment. Duncan's multiple range test at 5% level was used to differentiate means. (Snedecor and Cochran, 1980).

Influence of vessel and medium types on regeneration capacity of stored Sucarri orange shoots after different periods

Every 12 months, the recovery rate after storage was determined by estimating the number of shoots, leaves and length of shoots after 4weeks of subculture onto fresh basic medium. The stored shoots were taken out from the storage containers and placed on fresh medium in normal vessel culture (200mlGJ.). The basic salts and vitamins of MS medium supplemented with (mgl^{-1}) 0.2 BA+0.5 IBA was used. One replicate (three multiplied shoots) from each treatment was tested every six months. The shoots were taken out from storage conditions and divided into single shoot then cultured in 200ml GJ. Each treatment consisted of three replicates; and three shoots in each replicate. The number of shoots, leaves and length of shoots were recorded after subculture for 4 weeks .Three subcultures were performed for the recovery experiment.

Results and Discussion

Influence of culture vessel type and medium composition on the survival percentage of" Succari " orange cv original shoots during in vitro storage

Table 1 illustrated that the test tubes gave the highest mean survival % (85.16), without significance among all culture vessels. Concerning media composition data, (MSF) gave the highest significant mean survival (91.65) compared with the other media composition. The interactions between the two studied variables revealed that (MSF) medium in 200ml GJ. gave the highest survival % (100) while the lowest survival %was obtained with (MSH) medium in (GT. 25x80 mm) (66.62). After 24 months, survival percentages were decreased. However, there were high values in (GT. 25x80, GT. 25x150 mm), CF. and TT. (62.92, 62.92, 57.36 and 55.51), respectively with insignificant difference among them. The lowest mean was obtained in 350ml GJ. (38.85). Concerning the effect of media treatments data , MSF gave the highest survival % (61.99) with insignificant difference with (½MSF).The interactions between the two studied variables revealed that MSF gave the highest significant value in (GT. 25x80 , GT. 25x150 mm) and CF. (72.18), while the lowest mean was obtained in 350ml GJ. (33.3).

After 36 months of storage, the highest significant survival mean was recorded with (GT. 25x80 mm and 25x150 mm) (40.70), the lowest significant value was in 350ml GJ. (20.35). Concerning the medium treatments, MSH recorded the highest significant value (37.0), while the lowest significant value was for MSH (28.68). The difference between MSF and ½ MSF or between ½ MSF and MSH was insignificant. The interactions between the two studied variables revealed that MSF in (GT. 25x150 mm) and CF., also ½MSF with (TT. 15x150) and (GT.25x80 mm) gave the highest survival % (44.4), while the lowest mean was obtained in 350ml GJ. filled with MSH. In brief, it could be concluded that using 200ml GJ. as a culture vessel with MSF led to 100% survival after 12 months storage. However, the best survival was shown in GT. filled with ½MSF after 36 months (44.40%) and the survival was decreased. It would be advisable to terminate storage after 24 months and renew the medium to achieve short term storage. This was supported by the work of Mullin and Schlegel (1976) on strawberry plantlets which stored for up to 6 years in sterile cultures without subculture but with the addition of 1 or 2 drops of sterilized medium to the culture vessels every 12 months to extend the storage period without decrement of survival.

Influence of culture vessel type and medium composition on length {cm} of original shoots of "Succari " orange during in vitro storage

Table 2 illustrated that after 12 months the highest shoot length mean was obtained when using (CF.) and 200ml GJ. (3.92 and 3.70) without significancy between them. The lowest mean shoot length (2.72 and 2.83) was obtained in 350ml GJ. and GT.25x80 mm without significant difference between them. Regarding the effect of medium treatments data, ½MSF gave the highest significant mean length of shoots (3.69); while the MSH medium gave the lowest significant mean (2.79). The interactions between the two studied variables revealed that the MSF in CF. gave significantly the greatest shoot length (4.92) followed by ½MSF in 200ml GJ. or CF. (4.66 and 4.55) with insignificant difference among the three treatments.

TABLE 1. Effect of culture vessel type and media composition on survival percentage of Succari orange shoots during *in vitro* storage.

Treatments	Test tube (15x150mm)	Glass tube (25x80mm)	Glass tube (25x150mm)	Jar200ml (50x85mm)	Jar350ml (60x110mm)	Conical flask(100ml)	Mean
Storage periods (12months)							
MSF	94.43ab	88.87a-c	88.87a-c	100.0a	83.30a-c	94.43ab	91.65A
1/2MSF	88.87a-c	72.18bc	66.63c	77.73a-c	72.17bc	72.17bc	74.96B
MSH	72.17bc	66.62c	77.73a-c	72.18bc	88.87a-c	83.30a-c	76.81B
Mean	85.16A`	75.89A`	77.74A`	83.31A`	81.44A`	83.30A`	
(24 months)							
MSF	61.07a-c	72.18a	72.18a	55.5a-c	38.85bc	72.18a	61.99A
1/2MSF	61.07a-c	66.63ab	55.52a-c	49.96a-c	44.40a-c	44.40a-c	53.66AB
MSH	44.40a-c	49.95a-c	61.07a-c	38.85bc	33.3c	55.5a-c	47.18B
Mean	55.51A`	62.92A`	62.92A`	48.10AB`	38.85B`	57.36A`	
(36 months)							
MSF	35.85ab	38.85ab	44.40a	33.30a-c	22.20bc	44.40a	37.0A
1/2MSF	44.40a	44.40a	38.85ab	27.75a-c	22.20bc	27.75a-c	34.23AB
MSH	33.30a-c	38.85ab	38.85ab	22.20bc	16.65c	22.2bc	28.68B
Mean	38.85A`	40.70A`	40.70A`	27.75BC`	20.35C`	31.45C`	

TABLE 2. Effect of culture vessel type and media composition on length (cm) of Succari orange original shoots during *in vitro* storage.

Treatments	Test tube (15x150mm)	Glass tube (25x80mm)	Glass tube (25x150mm)	Jar200ml (50x85mm)	Jar 350ml (60x110mm)	Conical flask(100ml)	Mean
			Storage periods (12months)				
MSF	3.96b	2.31d	2.55cd	4.00b	2.30d	4.92a	3.34B
1/2MSF	3.88b	2.44cd	2.92c	4.66a	3.66b	4.55a	3.69A
MSH	2.25d	3.75b	3.83b	2.44cd	2.19d	2.30d	2.79C
Mean	3.37B`	2.83D`	3.10C`	3.70AB`	2.72D`	3.92A`	
			(24 months)				
MSF	4.19cd	3.03f	3.50e	4.17cd	2.92fg	5.08a	3.81B
1/2MSF	4.22cd	3.83de	4.50bc	5.33a	4.00cd	4.91ab	4.47A
MSH	2.25h	4.83ab	4.50bc	2.53f-h	2.66f-h	2.49gh	3.21C
Mean	3.55B`	3.89A`	4.17A`	4.01A`	3.19C`	4.16A`	
			(36months)				
MSF	4.16a-d	3.03c-f	3.50b-e	4.16a-d	1.92fg	5.08a	3.64A
1/2MSF	4.22a-c	3.92a-d	4.58ab	4.50a-c	2.66d-g	4.08a-d	3.99A
MSH	2.24e-g	5.00ab	4.42a-c	1.66fg	1.25g	1.75fg	2.72B
Mean	3.45A`	3.98A`	4.16A`	3.45A`	1.94B`	3.64A`	

Means followed by the same letters are not significantly different from each other at 5%level.
M SF... Full strength of Murashige &Skoog free growth regulators medium.
1/2MSF... Half strength of Murashige &Skoog free growth regulators medium.
MSH ... Full strength of Murashige &Skoog+(mg/l)1.0BA+0.4IBA+126phloroglucinol.

After storage for 24 months, the highest shoot length mean was obtained in GT.25x150, CF., 200ml GJ. and GT.25x80mm (4.17, 4.16, 4.01 and 3.89), respectively without significancy among them. The lowest shoot length mean was obtained in 350ml GJ. (3.19). Regarding the effect of medium treatments , ½MSF gave the highest shoot length mean (4.47), while MSH significantly gave the lowest shoot length mean (3.21). The interactions revealed that ½MSF in 200ml GJ. gave the highest shoot length (5.33) followed by CF. filled with MSF (5.08) and GT.25x80mm with MSH (4.83) with insignificant difference among them. The lowest shoot length was obtained in TT. filled with MSH (2.25). After storage for 36 months, the highest shoot length mean was obtained in GT.25 x 150mm (4.16) compared to other containers GT.25 x 80mm, CF., TT. and 200ml GJ. (3.98, 3.64, 3.45 and 3.45), respectively without significancy among them. The lowest shoot length mean was obtained in 350ml GJ. (1.94). It is worthy mentioning that in this period the cultures suffered from death and it was particularly obvious for cultures stored in 350ml GJ. , this led to a significant lower length compared to other treatments. Regarding the effect of medium treatments, ½MSF and MSF gave the highest mean shoot length (3.99 and 3.64) without significancy between them. MSH significantly gave the lowest shoot length mean (2.72). The interactions revealed that MSF in CF. gave the highest shoot length (5.08) followed by MSH in GT.25 x 80mm (5.00) with insignificant difference. It is also clear that no significant differences were obtained by TT., GT.25x80, GT.25x150, 200ml GJ. and CF. filled with ½ MSF. The lowest shoot length was obtained in 350ml GJ. filled with MSH (1.25). In this respect, it could be concluded that using GT.25 x 80mm and 350ml GJ. as a culture vessel with MSF showed the lowest shoot length after 12 months. The same trend was obtained after 24 and 36 months for 350ml GJ. However, by looking back to Table 1, it could be realized that the lower growth rate did not lead to high survival as expected .This results for 350ml GJ. could be attributed to the big exposed surface which permits high desiccation of medium.

Influence of culture vessel type and medium composition on average number of leaves /shoot of "Succari" orange during in vitro storage

Table 3 illustrated that, after 12 months storage, the highest mean number resulted in 200ml GJ. and CF. (25.17 and 24.22), respectively without significancy between them, whereas, the lowest rates were achieved in (GT.25 x 80 and 25 x 150mm) (15.50 and 16.44) with insignificant difference. In addition MSH showed significantly high mean number of leaves compared to the other media (22.79). The interactions showed that the highest significant number of leaves occurred in 200ml GJ. filled with MSH or ½MSF (26.0 and 25.83), while the lowest significant number of leaves obtained in GT.25 x 80mm filled with ½MSF (13.83). After storage for 24 months, the highest significant mean number of leaves was recorded in 200ml GJ. (30.06), while the lowest number of leaves was obtained in GT.25 x 80mm,TT and GT.25 x 150mm (21.66, 22.48 and 22.50), respectively with insignificant differences. Concerning the effect of medium treatments, MSH gave the highest significant mean leaf number (27.59). The interactions between the studied variables showed that the highest number of leaves occurred in 200ml GJ. filled with MSH (,33.33), whereas the lowest rates were achieved in GT 25 x 80 mm and TT. filled with ½MSF (18.67 and 17.89), respectively.

TABLE 3. Effect of culture vessel type and media composition on average number of leaves of Succari orange shoots during *in vitro* storage.

	Storage time (12months)						
Treatments	**Test tube (15x150mm)**	**Glass tube (25x80mm)**	**Glass tube (25x150mm)**	**Jar 200ml (50x85mm)**	**Jar 350ml (60x110mm)**	**Conical flask(100ml)**	**Mean**
MSF	24.33ab	14.67gh	15.50f-h	23.67ab	20.50cd	24.33ab	20.50B
1/2MSF	17.00e-g	13.83h	14.33gh	25.83a	21.67bc	23.83ab	19.42B
MSH	24.55ab	18.00d-f	19.50c-e	26.00a	24.17ab	24.50ab	22.79A
Mean	21.96B`	15.50C`	16.44C`	25.17A`	22.11B`	24.22A`	
	(24 months)						
MSF	25.00b-e	21.33ef	19.50f	27.67b-d	25.67b-d	26.33b-d	24.25B
1/2MSF	17.89f	18.67f	21.17ef	29.17b	23.83de	28.33bc	23.18B
MSH	24.55c-e	25.00b-e	26.83b-d	33.33a	27.67b-d	28.17b-d	27.59A
Mean	22.48C`	21.66C`	22.50C`	30.06A`	25.72B`	27.61B	
	(36months)						
MSF	25.00a-c	23.83a-c	20.67a-c	27.67a	17.33a-c	26.33ab	23.47A
1/2MSF	17.89a-c	21.00a-c	21.83a-c	25.17a-c	16.00a-c	25.00a-c	21.15A
MSH	24.55a-c	26.67a	27.83a	24.00a-c	13.67c	13.83bc	21.76A
Mean	22.48A`	23.83A`	23.44A`	25.61A`	15.67B`	21.72A`	

After 36 months storage, the highest mean number of leaves was obtained in 200ml GJ. (25.61) without significant difference between other containers except 350ml GJ. which recorded the lowest significant mean (15.67). The lower value of leaf number could be attributed to the high mortality of the stored shoots in this vessel. Regarding the effect of media treatments, there were insignificant differences among all types of media although the highest mean was recorded with MSF (23.47). The interactions revealed that MSH medium in GT.25x 150mm and GT.25x80mm vessels gave the highest number of leaves (27.83 and 26.67) without significant difference between them, while the lowest number of leaves was in 350ml GJ. filled with MSH (13.67). The obtained results emphasized the effect of medium composition and culture vessel types during different periods of storage and its relation with the growth rate of cultures. After 12 and 24 months the MSH gave the highest number of leaves which reflected on the high growth rate, while, after 36 months the growth rate decreased in this medium due to the decrement of survival. The trend in shoot length (Table 2) paralleled with the trend of the number of leaves, also, the number of leaves seems to decrease after 36 months as a result of survival decrement. From the inspection carried out every 6 months it was clear that cultures demonstrated lower growth rate (length and proliferation) after 30-36 months. Besides health and viability of shoots were less compared with previous periods of storage.

Influence of culture vessel type and medium composition on the number of new shoots of "Succari" orange during in vitro storage

Table 4 illustrated that after 12 months storage, the highest mean number of new shoots was obtained in 200ml GJ. and CF. (3.36 and 3.35) without significant difference between them, while the lowest mean number of new shoots was obtained in GT.25x80mm and 25x150mm (1.96 and 2.08) without significant difference between them. Concerning the effect of media, MSH medium gave the highest significant mean number of new shoots (3.41) and the lowest mean was obtained in ½ MSF medium (1.77). The interactions revealed that MSH in CF. and 200ml GJ. gave the highest number of new shoots without significant difference between them (4.05 and 3.93), while the lowest number of proliferated shoots was obtained in both GT. 25x150mm and 25 x 80mm filled with ½MSF (0.88 and 0.93), respectively. After storage for 24 months the highest significant mean of new shoots was obtained in 200ml GJ. and CF. (3.84 and 3.74) with insignificant difference between them. The lowest significant mean number of new shoots was obtained in GT. 25x80mm (2.43).Concerning the effect of medium treatments data showed the same trend as after 12 months storage. The interactions also revealed similar trend as after 12 months. After storage for 36 months, the highest significant mean number of new shoots was obtained in 200ml GJ. and CF. and the differences were insignificant compared with other containers except 350ml GJ. which recorded the lowest significant mean (1.72). Also, MSH and MSF media gave high mean of new shoots without signifcancy (3.11 and 3.07). The interactions revealed that MSF in CF. gave the highest number of new shoots (4.33), the lowest number of new shoots was obtained in GT.25 x 80mm and GT.25 x150mm which was filled with ½MSF (0.98 and 1.42). It was clear from the data that on the long run of the experiment MSF medium began to affect the number of shoots as MSH which was effective

through 12 and 24 months although the difference was insignificant after 36 months. In general, MSH recorded the highest significant mean number of shoots during different storage periods. Also, the large volume containers (200 ml GJ. and CF.) allowed the production of new shoots rather than the small volume containers during the period of 12 and 24 months except for 350ml GJ, while after 36 months, the differences among vessel types were insignificant except for 350 ml GJ which recorded the lowest value.

Influence of culture vessel type and medium composition on the length (cm) of "Succari" orange proliferated shoots during in vitro storage

Table 5 presented the effect of different vessel and medium types on the length (cm) of proliferated shoots during storage periods (12, 24 and 36 months). After 12 months in storage, the highest significant mean length of the new shoots was recorded in 200ml GJ. and CF. (2.72 and 2.59) without significant difference between them, while the lowest significant mean length of new shoots was achieved in TT. (1.71). Regarding the effect of media treatments, MSF medium gave the highest mean length of new shoots (2.47) followed by MSH medium (2.32) with insignificant difference, while the lowest value was recorded by ½ MSF (2.04). The interactions between the two studied variables showed that MSF in 200ml GJ. and CF. gave high length of new shoots (3.67 and 3.50), respectively, as well as MSH in GT. 25x80 and 25x150mm (3.67 and 3.42) with insignificant difference among the four treatments, while, the lowest significant mean was obtained in GT. 25x150mm filled with ½ MSF (0.85). Similar trend in the specific effect of culture vessel, media and in the interaction after 12 months was true after 24 months storage. After storage for 36 months, the highest significant mean length of new shoots was recorded in GT. 25x150mm (2.78) with insignificant difference when compared with mean length in other containers except TT. which showed the lowest significant mean (1.78). Concerning the effect of medium treatments, MSF gave the highest significant mean (2.90) whereas ½ MSF recorded the lowest mean (1.94) with insignificant difference with MSH (2.77). The interactions showed that MSF in CF gave high length of new shoots (4.25) followed by MSH in GT. 25x80mm and GT. 25x150mm without significant difference. The lowest average length was obtained in ½MSF in GT. 25x150mm (1.08). Using the same container GT. 25x150mm and MSH medium gave the highest length (4.16). The proper culture vessels with high survival percentage for storage differed after different periods of storage. As example during 12 and 24 months of storage, CF. and 200ml GJ gave the highest length, while at 36 months, GT.25x150 and 25x80mm gave higher value compared with the last period.

From the overall results it can be concluded that the big volume containers allowed the increase of length and number of shoots especially when filled with MSF and MSH (except 350ml GJ) in which large exposed surface caused early dryness of the medium while when ½MSF was used this effect was rather insignificant. From the storage point of view, the lower proliferation rate either represented by length or number of proliferated shoots was related to TT. and GT vessels. during most periods of storage , led to a higher survival *in vitro* after 3 years of storage *in vitro* .

TABLE 4. Effect of culture vessel type and media composition on average number of new Succari orange shoots during *in vitro* storage.

Treatments	Test tube (15x150mm)	Glass tube (25x80mm)	Glass tube (25x150mm)	Jar200ml (50x85mm)	Jar350ml (60x110mm)	Conical flask(100ml)	Mean
			Storage periods (12months)				
MSF	1.97fg	2.14e-g	2.61de	3.58ab	2.42d-f	3.66ab	2.73B
1/2MSF	1.89g	0.93h	0.88h	2.58de	2.00fg	2.33d-g	1.77C
MSH	3.66ab	2.83cd	2.75d	3.93a	3.25bc	4.05a	3.41A
Mean	2.50B`	1.96C`	2.08C`	3.36A`	2.56B`	3.35A`	
			(24 months)				
MSF	1.97g	2.80e	3.00de	4.17ab	3.00de	4.33a	3.21B
1/2MSF	2.60ef	0.98h	1.42h	3.03de	2.17fg	2.61ef	2.13C
MSH	3.66bc	3.50cd	3.83a-c	4.31a	3.42cd	4.27a	3.83A
Mean	2.75B`	2.43C`	2.75B`	3.84A`	2.86B`	3.74A`	
			(36 months)				
MSF	1.97f-h	2.80b-g	3.00a-f	4.16ab	2.16d-h	4.33a	3.07A
1/2MSF	2.61c-g	0.98h	1.42gh	2.44c-h	1.42gh	2.11e-h	1.83B
MSH	3.66a-d	3.58a-e	3.83a-c	3.08a-f	1.58f-h	2.88a-g	3.11A
Mean	2.75A`	2.46AB`	2.75A`	3.23A`	1.72B`	3.11A`	

Means followed by the same letters are not significantly different from each other at 5%level.
M SF... Full strength of Murashige &Skoog free growth regulators medium .
1/2MSF... Half strength of Murashige &Skoog free growth regulators medium.
MSH ... Full strength of Murashige &Skoog+(mg/l)1.0BA+0.4IBA+126phloroglucinol.

TABLE 5. Effect of culture vessel type and media composition on average length (cm) of new Succari orange shoots during *in vitro* storage.

Treatments	Test tube (15x150mm)	Glass tube (25x80mm)	Glass tube (25x150mm)	Jar200ml (50x85mm)	Jar 350ml (60x110mm)	Conical flask(100ml)	Mean
			Storage periods (12months)				
MSF	1.11hi	2.00d-g	2.16c-e	3.67a	2.41c-e	3.50a	2.47A
1\2MSF	2.39c-e	1.02hi	0.85i	2.58bc	2.92b	2.47b-d	2.04B
MSH	1.64g	3.67a	3.42a	1.91e-g	1.49gh	1.80fg	2.32A
Mean	1.71C`	2.23B`	2.15B`	2.72A`	2.27B`	2.59A`	
			(24 months)				
MSF	1.33h	2.67c-e	3.08b-d	3.92a	3.33b	4.25a	3.10A
1/2MSF	2.38ef	1.08i	1.08i	3.17bc	3.42b	2.61de	2.29C
MSH	1.64gh	4.41a	4.08a	2.19ef	2.25ef	2.05fg	2.77B
Mean	1.78C`	2.72B`	2.75B`	3.09A`	3.00AB`	2.97AB`	
			(36 months)				
MSF	1.33ef	2.66cd	3.08bc	3.92ab	2.16c-f	4.25a	2.90A
1/2MSF	2.38c-e	1.15ef	1.08f	2.66cd	2.25c-f	2.11c-f	1.94B
MSH	1.63ef	4.42a	4.16ab	1.42ef	1.25ef	1.27ef	2.36B
Mean	1.78B`	2.74A`	2.78A`	2.66A`	1.89A`	2.55A`	

Means followed by the same letters are not significantly different from each other at 5%level.
M SF... Full strength of Murashige &Skoog free growth regulators medium.
1/2MSF... Half strength of Murashige &Skoog free growth regulators medium.
MSH ... Full strength of Murashige &Skoog+(mg/l)1.0BA+0.4IBA+126phloroglucinol.

Influence of vessel and medium types on regeneration capacity of stored "Sucarri" orange shoots after different periods of storage

In this experiment, shoots which have recorded the highest survival % during storage, were taken out from the storage vessels and divided into single shoot, then placed on fresh medium MS full strength supplemented with (mgl^{-1}) 0.2 **BA** +0.5 IBA in normal vessel culture (200ml GJ.). Each selected treatment was tested after 12, 24 and 36 months storage .

Data given in Table 6 showed the effect of transferring shoots to fresh medium after different storage periods in order to study the regeneration capacity of the stored shoots. All transferred shoots recorded (100%) survival in all subcultures. The shoots assumed normal growth within four weeks and the rate of growth increased through three subcultures. After 12 months storage, shoots were taken out from TT, 200ml GJ. and CF. vessels were filled with MSF (Table 6). Concerning the effect of different vessel types, data revealed that there was no significant difference in mean number of shoots and leaves and significant difference in mean shoot length resulted from CF. and 200ml GJ. compared to TT. Regarding the number of subcultures, the third subculture gave the highest significant length of shoots (1.4cm), the highest number of shoots (1.92) and the highest number of leaves (20.0). The interactions between the two studied variables showed that the shoots taken from 200ml GJ. during the third subculture gave the highest significant number of shoots (2.0) and number of leaves (20.33) but without significant difference with other treatments. The shoots taken from CF. recorded the highest significant length during the 3rd subculture. After 24 months, the selected transferred shoots were from CF., GT. 25x80mm and GT. 25x150mm which was filled with MSF, also from GT. 25x150mm filled with ½MSF (Table 6). Insignificant difference in mean length and number of shoots resulted when the stored shoots transferred from the different vessel types to fresh conditions. However, high significant mean number of leaves per shoot resulted on shoots taken from GT. 25x150mm with MSF (17.11). As for the effect of subcultures, the same trend was observed as after 12 months recovery. The interactions between the two studied variables revealed that the shoots taken from GT.25x80mm with MSF gave the highest length of shoots (1.87) during the third subculture without significant difference with other treatments in the three subcultures, while, the shoots taken from GT.25x150mm with ½MSF recorded the highest insignificant number of proliferated shoots (2.33) during the 3rd subculture. Concerning the number of leaves, the shoots taken from GT.25x150mm with MSF gave the highest insignificant value (23.67) during the 3rd subculture. Similar trend was clear in the recovery of plants after 36 months. The shoots taken out from CF. and GT.25x150mm with MSF or from TT. and GT.25x80mm with ½ MSF showed insignificant differences in mean length and number of proliferated shoots while the mean number of leaves was significantly higher by TT and ½MSF. In general, the growth rate was slower in the first subculture then increased gradually during second and third subcultures. The highest rate of growth was recorded in the 3rd subculture. The effect of vessel types on the average length, shoot number, was insignificant, while the effect on leaf proliferation was significantly recorded by GT.25x150mm after 24 months and by TT. after 36 months. In brief, the cultures that demonstrated lower growth rate (length and number of shoots) and high survival percentage were clear in TT, GT25x80, 25x150mmand CF with both MSF or ½MSF medium.

TABLE 6. Recovery of stored Succari orange shoots after different periods of storage.

Treatments	Shoot length				Number of shoot				Number of leaves				Survival
	Sub. 1	Sub. 2	Sub. 3	Mean	Sub. 1	Sub. 2	Sub. 3	Mean	Sub. 1	Sub. 2	Sub. 3	Mean	%
After 12 months													
TT+MSF	0.73e	1.03cd	1.26b	**1.01B**	0.88d	1.66a-c	1.88ab	**1.47A**	10.0c	14.33bc	19.67a	**14.67A**	100a
200mljar+MSF	0.86de	1.06c	1.43ab	**1.12A**	0.88d	1.55bc	2.00a	**1.48A**	9.33bc	14.33bc	20.33a	**14.66A**	100a
C.F+MSF	0.86de	1.07c	1.50a	**1.14A**	0.77d	1.33c	1.88ab	**1.33A**	12.0bc	15.33ab	20.0a	**15.78A**	100a
Mean	**0.82C`**	**1.05B`**	**1.40A`**		**0.84C`**	**1.51B`**	**1.92A`**		**10.44C`**	**14.66B`**	**20.0A`**		**100A`**
After 24 months													
GT25x80+MSF	0.80a	1.30a	1.87a	**1.32A**	0.88e	1.55cd	1.99a-c	**1.47A**	9.66e	14.0cd	22.0a	**15.22B**	100a
GT25x150+MSF	0.90a	1.06a	1.26a	**1.07A**	0.77e	1.66bc	2.11ab	**1.51A**	12.33c-e	15.33c	23.67a	**17.11A**	100a
C.F+MSF	0.93a	1.16a	1.50a	**1.20A**	0.88e	1.55cd	2.22a	**1.55A**	10.0e	13.67cd	18.67b	**14.11B**	100a
GT25x150+1/2MSF	0.83a	1.33a	1.60a	**1.25A**	1.11de	1.55cd	2.33a	**1.66A**	11.0de	12.67c-e	21.67a	**15.11B**	100a
Mean	**0.86B`**	**1.22AB`**	**1.55A`**		**0.91C`**	**1.58B`**	**2.16A`**		**10.75C`**	**13.92B`**	**21.50A`**		**100A`**
After 36 months													
CF+MSF	0.86f	1.03d-f	1.40a-c	**1.10A**	0.77c	1.33b	1.88a	**1.33A**	11.33e	16.33d	22.0ab	**16.56B**	100a
GT25x150+MSF	0.86f	1.16c-e	1.43ab	**1.15A**	0.88bc	1.33b	1.99a	**1.40A**	12.67e	12.33e	22.33ab	**15.78B**	100a
TT+1/2MSF	0.86f	1.16c-e	1.50a	**1.17A**	0.88bc	1.33b	1.99a	**1.40A**	12.67e	18.0cd	24.33a	**18.33A**	100a
GT25x150+1/2MSF	0.93ef	1.23b-d	1.56a	**1.24A**	0.88bc	1.33b	1.99a	**1.40A**	13.0e	16.0d	20.33bc	**16.44B**	100a
Mean	**0.88C`**	**1.15B`**	**1.47A`**		**0.85C`**	**1.33B`**	**1.96A`**		**12.42C`**	**15.67B`**	**22.25A`**		**100A`**

Means followed by the same letters are not significantly different from each other at 5%level.
M SF.:. Full strength of Murashige &Skoog free growth regulators medium.
1/2MSF... Half strength of Murashige &Skoog free growth regulators medium

The obtained results emphasized the effect of medium composition and culture vessel types on the storage of citrus "Succari" orange cultures *in vitro*. In the present study, the main target was more conducive to arrive to the lowest growth rate and high survival by using minimal growth medium and without subculturing for longer periods. Concerning the vessel type, it could be realized that the lowest survival after 24 and 36 months occurred when using 350mlGJ filled with MSH medium, which might be attributed to the high desiccation of media from the big surface of the vessel . The same suggestion was reported on tiller explants of *Lolium multiflorm* and apple species shoot cultures grown in large vessels compared to test tubes (McClelland and Smith, 1990). Similar results were obtained by Wanas (1987) and Kozai *et al.* (1995) where pear shoot tip cultures and potato gave better quantity and quality of proliferated shoots in glass tubes (150 x 25mm) rather than polypropylene tubes (100 x 15mm). George & Sherrington (1984) and Debrgh (1988) attributed the effect of vessel type or volume on the growth and morphogenesis *in vitro* to different concentration levels of carbon dioxide, ethylene and other volatiles in the air space within the containers. The notes were shown on morphology of cultures (decrease of the level of media, the viability of shoots, besides the growth rate and the poor growth of shoots).In this respect, the water relations may be the most important factor. As stated by Kozai *et al.* (1986) and DeGryze *et al.* (1995), the factors that control water keeping in the tissue culture vessel fall under one of the following categories: the composition of the culture medium, the characteristics of the container, the environmental conditions and/ or the quality of the explant. Also, the type of closures for the vessels and degree of exchange between inside and outside the container do have a considerable influence on the water keeping of tissue cultured plants. Depending on the material container is made of and its construction, different possibilities of exchange between the container atmosphere and the environment existed (DeGryze *et al.*, 1995). Another point of interest is that highest survival percentage in this study was achieved during the first period of storage (12 months) as in Table 1. The survival percentage however, was decreased during the second and third periods (24 & 36 months). It would be wise to renew the medium by the addition of 1 or 2 drops of sterilized medium to the culture vessels after 12 months to extend the storage period without decrement of survival. Addition of medium as a storage method was achieved with strawberry plantlets which were stored for up to 6 years as meristem plantlets in sterile cultures without subculture or transfer of the explant to fresh medium (Mullin and Schlegel, 1976). The suitability of higher temperatures at the range of 24-26c° for shoot storage was confirmed by Kartha *et al.* (1981) and Roca *et al.* (1982) on coffee (*Coffea arabica* L.) and Cassava. Also, Marin and Duran Vila (1991) reported on " Pineapple "sweet orange plantlets and nodal stem segments which were kept under normal conditions for 12 months before transferring to fresh medium. Besides Wanas (1999) found that the cultures of citrus species maintained viable *in vitro* at 24°C plus low light intensity (20 $\mu Em^{-2}s^{-1}$) for 12-16 months without subculturing, the survival percentage (100) was recorded for Rangpur lime after 16 months storage.

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بعض العوامل المؤثرة على التخزين المعملى لمزارع البرتقال السكرى في ظروف النمو العادية

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تهدف هذه الدراسة نحو إمكانية استخدام تقنية زراعة الأنسجة في الحفظ المعملى للبرتقال السكرى. وفيها تم دراسة كلا من أثر وعاء التخزين ونوع البيئة المستخدمة للتخزين تحت الظروف العادية (٢٥±٢°م) وإضاءة لمدة ١٦ ساعة وإظلام لمدة ٨ ساعات وشدة الإضاءة ٢٥٠٠ لاكس.

وتم التخزين لفترات زمنية مختلفة حتى (٣٦ شهراً) بدون نقل النموات لبيئة جديدة . وكانت البيئات المستخدمة هى بيئة أملاح موراشيج وسكوج كاملة قوة الأملاح وهى خالية من الهرمون (MSF) وبيئة موراشيج وسكوج نصف قوة الأملاح وخالية من الهرمون (½MSF) وبيئة موراشيج وسكوج مضافاً لها ٠,٤ ملجم أندول حامض البيوتريك ، ١ ملجم بنزيل أدينين ، ١٢٦ملجم فلورجلوسينول (MSH) والأوعية المستخدمة هى أنابيب زجاجية ١٥ × ١٥٠ مم (T.T) ، أنابيب زجاجية ٢٥ × ٨٠ مم ، ٢٥ × ١٥٠ مم (G.T) وبرطمانات (٢٠٠ مل ، ٣٥٠ مل) (GJ) ودوارق مخروطية ١٠٠ مل (CF). وكانت أعلى نسبة بقاء مع الأنابيب ٢٥ × ١٥٠ مم والدورق المخروطى ١٠٠ مل والمحتوية على (MSF) وكذلك مع الأنابيب ١٥ × ١٥٠ مم ٢٥ × ٨٠ مم المحتوية على (½MSF). في حين أن الأوعية الأخرى (برطمان ٢٠٠ مل ، ٣٥٠ مل) سجلت أقل نسبة بقاء خلال ٣٦ شهراً (مدة التخزين) ولا سيما مع بيئة (MSH) وذلك نتيجة جفاف البيئة من هذا المسطح الكبير. وتحقق أعلى طول للنبات أو للنمو الأصلى مع الدورق ١٠٠ مل المحتوى على (MSF) وأقل طول مع البرطمان ٣٥٠ مل المحتوى على (MSH).

وكان أعلى معدل للأوراق المتكونة في الأنابيب ٢٥ × ٨٠، ٢٥ × ١٥٠ مم المحتوية على MSH وكذلك في البرطمان ٢٠٠ مل المحتوى على (MSF) في حين أن أقل عدد للأوراق مع البرطمان ٣٥٠ مل المحتوى على (MSH) وكان أعلى عدد للنموات الجديدة المتكونة مع الدورق المخروطى ١٠٠ مل المحتوى على (MSF) وسجل البرطمان ٣٥٠ مل مع نفس البيئة أقل عدد للنموات.

وبالنسبة لصفة طول النموات الجديدة على النمو الرئيسى فقد سجل كلا من الدورق ١٠٠ مل المحتوى على MSF ، الأنابيب ٢٥ × ٨٠ مم المحتوية على MSH أعلى معدل لطول النموات وكان أقل معدل مع الأنابيب ٢٥ × ١٥٠ مم المحتوية على (½MSF).

وفي تجربة القدرة على استعادة النمو للنبات المخزنة كان معدل النمو بطئ في النقلة الأولى وتزايد تدريجياً خلال النقلة الثانية ثم الثالثة.