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INTRODUCTION OF GRAPES (*Vitis vinifera* L) INTO THE *IN VITRO* COLLECTION OF NATIONAL GENE BANK AND GENETIC RESOURCES OF EGYPT.

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Abstract: Eight Accessions of grape (*Vitis vinifera*) are introduced and kept *in vitro* in the National Gene bank and Genetic Resources of Egypt. The objectives of this study were to determine optimal method for rapid clonal propagation of two local Egyptian cultivars (Khalili and Edkawy) using apices and nodal cutting stem dissected from 9 old month grape plants grown in green house taken from mature field growth plants. The effects of two different introduction media for the micropropagation of different grape cultivars were studied. A factorial treatment with different combinations using 3 concentrations of MS (full,3/4 and half strength of MS), 3 concentrations of BAP (0.5 ,1.0,2.0 and 3.0 mg/ L) , 4 concentrations of IAA(0.1, 0.3, 0.5 and 1 mg / L) and one concentration of TDZ (0.05 mg/L), respectively were used for multiplication. The explants were *in vitro* rooted using medium supplemented with various combination and concentrations of MS, IAA, BAP and IBA, either with or without addition of charcoal. The best media for the multiplication rate of Khalili cultivar was achieved with ¾ MS supplemented with o.5 mg/L IAA and 1mg /L BAP, while the best medium for Edkawy cultivar was 1/2 MS supplemented with 0.5 mg /L BAP and 0.1 mg/L IAA. High root number was achieved with media contained full strength of MS supplemented with 2 mg/L IAA and 0.5 mg /L BAP for Khalili cultivar, whereas the high root number for Edkawy cultivar was obtained by media included full strength of MS added with 3 mg/L IAA and 0.5 mg /L BAP. The rooted plantlets were acclimated to *ex vitro* conditions, most of the rooted shoots survived when transferred to pots containing Peat moss and Vermiculate (1:1).

Keywords: grape, *Vitis vinifera, in vitro*, Gene bank, multiplication, rooting, acclimatization

Introduction

The genus *Vitis* is broadly distributed, largely between 25 and 50° N latitude in eastern Asia, Europe, the Middle East and North America. The genus *Vitis* divided into three important species and one hybrid group including *vinifera* L

Grapevines traditionally propagated from cutting of dormant one year old canes Dzazio *et al.*(2002) and the primary use of

tissue techniques was to the elimination of grapevine diseases

(Glazy, 1964 and Masahiko *et al* 1982).By using micropropagation techniques thousands of plants can be produced from single vine (Monette, 1988).

To date, the collection of vegetatively propagated plants (as grapes) has been preserved in the field or greenhouse. However, as for other vegetatively propagated crops, there is a risk of accumulative contamination by viruses and high labor intensity in the field. Therefore genebanks have started working on medium term conservation of this species by means of *in vitro* culture (Mix and Schittenhelm 1991). Therefore introducing of local and imported varieties to Egyptian National Gene Bank and Genetic Resources (NGBGR)is of great importance development of an *in vitro* collection and developed a method of *in vitro* propagation as a primary step before preservation.

Lee and Wetzstein (1990) studied the effect of cytokinin on elongation, prloliferation and total mass of shoot apices of grapevine cv. Summit and found that the best total shoot production was obtained with 10.0 µM BA, while shoot vigour was obtained on MS medium supplemented with 5.0 µM . Cha *et al.* (1991) found that the optimum range of nitrogen concentration for shoot growth of grapevine cv. Campbell Early was 75-100% of the full strength medium. Blazina *et al.* (1992) examined the nutritional requirement of (*Vitis vinifera* L. Zelen) from shoot tip meristems and achived regeneration by using 1/2 MS medium supplemented with 2% sucrose and 1.1 µM IAA. They also stated that MS medium supplemented with 3% sucrose, 0.7% agar, 5.0 µM benzyladenine and 1.1 µM IAA resulted in the greatest meristem proliferation. While, Gray and Benton (1991) regenerated axillary bud of 9 muscadine grape cultivars using 4 media formulation MS, ½ MS, C2D and WPM. First three media produced equivalent shoot proliferation rates, whereas WPM produced stunted shoots. They found that BA at 5.0, 10.0 and 20.0 uM or 5 µM TDZ produced the highest average number of cultured apex. Compton and Gray (1995) achieved 88% production of shoots from (Southern Home) hybrid grape which cultured on MS medium containing BA than those on medium with IAA, GA3 and kinetin (57%).El- Din *et al.* (1997) evaluated the propagation of 5 muscadine grapes in vitro and found that the best survival of explants was recorded for Regal on a medium containing BA at 2 mg /L. On the other hand, Minal *et al.* (2000) produced a tuft of multiple shoots on a medium containing BAP, and IBA.. Yuen Yan and Weifang (2001) reported that using BA at 1.0 or 2.0 mg /L produced 100% bud differentiation for 3 cultivars of grapes (Wuhebai jixin,

Meirenzhi and Yongyou F1). Also, Dzazio *et al.* (2002) established a protocol for micropropagation of 420/A grape rootstock using ½ MS supplemented with 1.0 µM BA. Meanwhile, Singh et al., (2004) using MS medium containing 4.0 mg /L BA + 0.2 mg/L NAA reported the best response (85.6%) for the culture initiation for grape Cv. Pusa Urvashi. Nalwade and Shitole (2004) used nodal cutting and axillary bud of (*V. vinifera*) cv. Tas-A- Ganesh on a medium consisting of 13.2 µM BA alone to obtained maximum no. of shoots (3.7+or – 0.48 per explant) while using combination of 11.0 µM BA+ 2.85 µM NAA induced healthy and staut shoots (4.1 per explant) indicating that is better than supplemented with BA alone.

Successful tissue culture method of propagation must result in establishment of derived plants in soil. So the function of rooting stage is to prepare the plantlets for transplanting and establishment outside the artificial closed environment of culture vessel. Murashige(1974). Lee and Wetzstein (1990) used IBA to enhance rooting by increasing rooting percentage and root number by plantlet. On the other hand, Gray and Benton (1991) used 1.0µM NAA which significantly increased all parameters measured. Al-Maarri and Al- Ghamdi (1995) cultured shoot tips of Banaty and Khalas cultivars on MS medium containing 6 g/L agar, 30/L g sucrose , 100 mg myo- inositol and 0.4 g thiamin, supplemented with 0.2 mg NAA, 0.4 mg GA3 and 1 mg/L BA. Greater than 80% rooting was achieved with 1/3 strength MS medium containing 0.6% agar and 2% sucrose supplemented with 100.0 mg – inositol, 0.4 mg thiamin and 0.2 mg IBA/l.. Moreover, El- Din *et al.* (1997) found that 0.5 mg/ L IBA was effective for promoting root initiation and development. Yuen Ya and Weifang (2001) reported that half strength MS contained IBA at 0.1 or 0.2 mg/ L produced rooting at rate of 84- 96% and rooting number of 5-7 per explant for 3 cultivars of grapes (Wuhebai jixin, Meirenzhi and Yongyou F1). Also, Singh *et al.* (2004) achieved rooted plants by using half strength MS contained IBA at 2.0 mg/ L + 200 mg / L activated charcoal.

Materials and Methods

In vitro propagation

Greenhouse –grown 9 old month potted plants with actively growing shoots 10 – 15 cm long were used as a source of materials for two local grapes cultivars (Khaliliy and Edkawy). After removing the leaves, shoots were put under running tap water for about one hour and then sterilized under laminar flow hood condition. Then surfacely sterilized with 70% ethanol for 1min followed by sodium hypochlorite solution was prepared using commercial bleach "Clorox" (5.25 % available chlorine) at 20 % concentration and the shoots were dipped for 20 min

and then rinsed three times with sterile distilled water. After the sterilization, stem nodes were cultured in establishment culture media. The pH of different media was adjusted to 5.7 before autoclaving at 100 K. pa (15 P.S.I) and 121°C for 20 minutes, then left to cool and harden. The cultures of different experiments were incubated at temperatures almost maintained between 25 ± 2 °C and photoperiods of 16 hour at day time and 8 hour night supplied by fluorescent lamp to provide light

Establishment stage

Each stem node explants from each cultivar was separately cultured in tubes (100 x25 mm) contained of full strength Murashige and Skoog (1962) (MS) plus 3% sucrose, 0.7% agar and supplemented with benzylaminopurine (BAP) at 0.0 (control) or 0.5 mgl^{-1} in combination with indole butyric acid (IBA) 2 mgl^{-1} or half strength Murashige and Skoog plus 2% sucrose, 0.7 % agar and supplemented with Indole acetic acid (IAA) at 2 mg/L. Survival percentages, shoot number and shoot length (cm) were determined after four weeks. The experiment was repeated three times and was arranged in a completely randomized design with 3treatments for each cultivar with four replicates (three explants for each replicate)

1-MS+ 3% sucrose (control)

2-MS+3% sucrose+2 mg/ L IAA +0.5 mg / L BAP

3- 1/2MS +2% sucrose + 2 mg / L IAA

Proliferation stage

Approximately uniform growing shoots 1.5 cm in length from both cultivars were aseptically transferred after four weeks to proliferation medium which was consisted of MS (full,3/4 and half strength of MS), 4 concentrations of BAP (0.5 ,1,2 and 3 mg/ L) , 4 concentrations of IAA(0.1, 0.3, 0.5 and 1 mg/ L) and one concentration of TDZ.(0.05 mg/L) .

The proliferated shoots were subcultured onto fresh medium three times, each of 4 weeks period. Survival percentage, average number and length (cm) of new proliferated shoots were recorded each subculture during the proliferation stage.

The experiment was arranged in a completely randomized design with 9 treatments for each cultivar with four replicates (three explants for each replicate). The best treatment which recorded the highest proliferation rate was used for this experiment in which the shoots were transferred into fresh medium every four weeks. Survival %, average number and length (cm) of new proliferated shoots were recorded for Khalili and Edkawy grapes cultivars.

1-¾ MS +2% sucrose +1 mg/ LBAP +1 mg/ L IAA	7-1/2 MS +2% sucrose +1 mg/ LBAP +0.5 mg/ L IAA
2- full MS +2% sucrose +1 mg/ LBAP +1 mg/ L IAA	8-1/2 MS +2% sucrose + 5µM TDZ
3-¾ MS +2% sucrose +2mg/ LBAP + 0.3 mg/ L IAA	11-3/4 MS +2% sucrose +1 mg/ L BAP +0.5 mg/ L IAA (khaliliy)
5-¾ MS +2% sucrose + 2mg/ LBAP + 0.5 mg/ L IAA	12-1/2MS +2% sucrose +0.5 mg/ LBAP +0.1 mg/ L IAA (Edkawy)
6- -¾ MS +2% sucrose + 3mg/ LBAP + 0.3 mg/ L IAA	

Rooting stage

Uniform proliferated shoots about 2 cm in length from the 3rd subculture were transferred to glass jars 350 mm filled with 25 ml of rooting medium which consisted of 1/2 strength,3/4 strength or full strength or MS medium plus 2% or 3% sucrose, 0.7 % agar and supplemented with IBA (2 and 3 mg/L) , IAA (0.5,1.5 and 3 mg/ L) , BAP (0.5 mg /L) (IAA or IBA + BAP) and with or without addition of activated charcoal. The following measurements were recorded after 8 weeks of culturing on rooting medium: rooting percentage, average number and length (mm) of roots.

8- full MS +3% sucrose + 1.5 mg/L IAA + 0.5BAP
7-full MS +3% sucrose + 3 mg/L IAA + 0.5BAP
9-1/2 MS +2% sucrose + 2 mg/L IBA + A.C
10- 1/2 MS +2% sucrose + 3 mg/L IBA + A.C
4 -3/4 MS +0.5 mg IAA

The experiment for each cultivar (Khalili and Edkawy) was arranged in a completely randomized design with four replicates; three explants each.

Rooted shoots (taken from the best treatment after the 3rd subculture) were rinsed carefully with sterile distilled water to remove adhering medium and put it in a fungicide (1.0 gl-1) for 2 min. before transplanting to plastic 10 x 15 cm. pots filled with a mixture of Vermiculate and Peat moss (1:1 by volume) or Vermiculate only and covered with clear plastic bags then maintained in greenhouse for 4 weeks with artificial lighting, and 80% relative humidity.

Split- split plot design was achieved to determine the effects of medium concentration and benzylaminopurine at different concentrations and TDZ. Analysis of variance was used for data analyses. Means separation was

conducted by the use of Duncan's multiple range test at 5% level of probability(Snedecor & Cochran , 1980)

Results and discussion

Effect of medium type and growth regulators (BA and IBA) levels on survival percentage and shoot length during establishment stage:

In this respect, the influence of 2 medium types (full MS and 1/2 MS) and cytokinins

effect 0.5 mg/L BA and auxin effect 2 mg/L IAA and their effect on survival %, shoot no., explant shoot length and leave no. are shown in Table(1).

Concerning the survival percentage and shoot number per explant, the obtained data exhibited no specific effect for both of culture media and cytokinin or auxin on survival percentage and shoot number per explant for both Khalili and Edkawy grape cultivars. Concerning shoot length and leaf number per cultured explant there was significant differences between studied media , obtained data exhibited that half MS was more effective from full MS media. Also, using sucrose 2% concentration showed superiority as compared to 3% concentration for both Khalili and Edkawy grape cultivars. As the same trend specific effect of growth regulators revealed that IAA at 2 mg /L exhibited the highest shoot length and leaf number than using IAA at 2 mg/ L plus 0.5 mg/L BA. The interaction among the three studied factors showed that half MS media plus 2% sucrose and supplemented with IAA at 2 mg/L recorded the highest significant shoot length (3.5 and 2.1mm) and leaf number (4.0 and 3.0) for both Khalili and Edkawy respectively.

These results were not in agreement with the results obtained with (Dzazio et al.2002, Singh et al. 2004 and AL Maari and AL Ghamdi 1995) who reported that the greatest growth of bud obtained with MS full strength media for 420-A rootstock, Pusa Urvashi grape cultivars and Banaty and Khalas cultivars. Also, AL Maari and AL Ghamdi (1995) mentioned that 3% sucrose was used for the initiation stage. Furthermore, EL-Dine et al. (1997) found best survival of explants was recorded for Regal grape cultivar on medium containing BA at 2 mg/L .

Effect of medium type and different cytokinine concentrations on survival %, shoot no. and shoot length (mm) during proliferation stage.

Data in Table (2) revealed that there were no significant differences among different media type concentrations (full MS, 3/4 MS and 1/2 MS) on the survival % for the two grape cultivars. Also , presented data during proliferation stage showed that insignificant differences among different cytokinines and auxins on the survival percentage.

As for the interaction between medium type, cytokinin and auxin concentration, insignificant differences were noticed among all tested treatments in survival % for each of Khalili and Edkawy grape cultivars (Figure 1).

Data in table (2) revealed that the highest significant number of new proliferated shoots for Khalili grape was noticed with 3/4 MS medium, while 1/2 MS medium gave the least value. Concerning the effect of BA and TDZ levels, there were significant differences between the different cytokinins and their concentrations. The highest significant number of shoots / explant (9.3) was achieved with 1.0 BA plus 0.5 IAA at (mg/L). Meanwhile, TDZ at 5.0 µM produced the lowest value of shoot number / explant (2.0) (Figure 1).

The interactions between the two studied factors showed that MS medium supplemented with 1.0 BA plus 0.5 IAA at (mg/L) recorded the highest significant number of shoot number/ explant (9.3).On the other hand, presented data of Edkawy cultivar showed that the

Table (1): Effect of medium type and (BA, IBA) combinations on survival % and shoot length (cm) for nodal explant cultures of Khalili and Edkawy grapes during establishment stage.

Parameter / Treatment	Survival%		Shoot no.		Shoot length(cm)		Leave no.	
	Khalily	Edkawy	Khalily	Edkawy	Khalily	Edkawy	Khalily	Edkawy
Control (MS + 3% sucrose)	90B	70 C	1.0 A	1.0 A	1.5 C	2.0 B	2.0 C	2.5 C
1-MS+3% sucrose+2 mg/ L IAA +0.5 mg / L BAP	100 A	100 A	1.0 A	1.0 A	2.0 B	1.0 C	3.0 B	2.0 C
2- 1/2MS +2% sucrose + 2 mg / L IAA +0.5 mg / L BAP	100 A	100 A	1.0 A	1.0 A	3.5 A	2.1 B	4.0 A	3.0 B

highest shoot / explant were obtained on 1/2 MS medium. Regarding to the effect of BA and TDZ levels, there were significant differences between the different cytokinins and its concentrations. The highest significant number of shoot / explant (8.3) was achieved 0.5 BA plus 0.1 IAA (mg/L). In the meantime BA at 1.0 mg/L plus IAA at 0.5 mg/L gave the least value (1.0). Table (2)

The interactions between the

two studied factors showed that 1/2 MS medium supplemented with 0.5 BA plus 0.1 IAA (mg/L) recorded the highest significant number of shoots / explant (8.3) (Figure 1).

With regard to shoot length per cultured explant as influenced by specific effect of kind of culture medium for Khalili cultivars, obtained data exhibited that 3/4 MS medium generally the most effective from 1/2 MS on Khalili grape cultivar. BA and TDZ levels showed significant differences. The highest shoot length (4.6 mm) was achieved with 1.0 BA plus 0.5 IAA (mg/L). While, 5.0 µM TDZ showed the lowest significant shoot length and the shoots were stunted (0.4). Table (2)

The interactions between the two studied factors showed that 1/2 MS supplemented with 1.0 BA plus 0.5 IAA (mg/L) recorded the highest significant average length.

In contrast, the Edkawy cultivar results showed that the specific effect of culture media was for 1/2 MS media. Specific effect of cytokinins added to culture media through proliferation stage, data obviously show the superiority of adding BA at 1.0 plus 0.5 IAA (mg/L). While, the lower shoot length was produced when used TDZ at 5.0 µM and the shoots were stunted.(Table 2)

The interactions between the two studied factors showed that 1/2 MS supplemented with BA plus 0.5 IAA (mg/L) scored the highest significant average length (10.3).

These results were in agreement with those of (Lee and Wetzstien 1990, Yuen Ya and Wiefang 2001, Bigger and Read 2002 and Gray and Beneton 1991) who mentioned that using 20 µM BA, 4 µM and 10 µM BA produced the highest average number of shoot per culture apex .Also, TDZ produced stunted shoots. Table (2) (Figure 1).

Blazina et al. (1992) mentioned that the best medium used for multiplication was MS supplemented with BA and IAA. Furthermore, Nalwade and Shitole (2004) found that using combination of BA and IAA induced healthy shoots (41shoot/ explant) indicating that this is better than supplementing with BA alone. Singh et al. 2004 and AL Maari and AL Ghamdi 1995) recommended that MS medium supplemented with 4.0, 1.0 mg/L BA respectively, gave the best response for proliferation stage. These results are in accordance with those obtained by other authors (Barlass and Skene 1980, Silvestroni 1981, Chee and Pool 1983, Safadi and Abu Irmaileh 1987) which suggested that 2.22 to 11.1 µM is the optimum concentration for the micropropagation of different plant materials of *Vitis spp.* Also, Ibanez et al, (2003) stated that BA produced the best results especially at 6.6 7 and 8.9 µM BA. Table (2)

The experiment described in this study set out to ascertain the type of cytokinin and its concentrations

which led to the greatest proliferation of shoots and formation of axillary bud microcuttings from in vitro grown plants of the table grapevine cultivars Khalili and Edkawy. The shoots developed were of good appearance with an intense green color and of uniform size for Khalili and Edkawy cultivars. The best Murashig and Skoog (MS) media concentration was 3/4 and of four cytokinins assayed, BA produced the best results especially at 1mg/L Plus 0.5 mg/L IAA for Khalili cultivar. On the other hand, half strength of MS was the best concentration for the proliferation of Edkawy cultivar,of the four cytokinins assayed, BA produced the results especially at 0.5/L plus 0.1 mg/L IAA. The optimum concentration of BA shows very slightly between cultivars and that it should be determined in each individual case. Despite the significant plant growth there was increase in negative symptoms such as callus formation at base of the explants and the vitrified, stunted and swollen appearanceof shoots with clustered axillary buds and reduced leaf expantion.

Effect of medium type and different cytokinin concentrations on survival %, shoot no. and shoot length (mm) during rooting stage.

Concerning the effect of auxin type and concentration on Khalili grape cultivar shoots rooting Table (3), revealed that the great rooting percentage (100%) was obtained by MS full strength medium with 3.0 mg/L IAA and 0.5 mg/L BAP. While the lowest rooting percentage was obtained by MS medium full strength supplemented with 2.0 mg/L IAA + 0.5 mg/L BAP.

Insignificant differences between MS + 1.5 mg/L IAA + 0.5 mg/L BAP, 1/2 MS + 2.0 mg/L IBA and 1/2 MS + 3.0 mg/L IBA were obvious for rooting % of Edkawy cultivar was higher at 2.0 mg/L IAA and 3.0 mg/L IAA with no significant differance between them. In contrast, using IAA at 1.5 mg/L, IBA at 2.0 mg/L did not give any root growth, Table (3) .While, the highest number of roots occurred with IAA at 3.0 mg/L followed by IAA at 1.5 mg/L and IBA at 2.0 mg/L with no significant differance between them. The lowest roots number was recorded by IBA at 3.0 mg/L (for Khalili cultivar). On the other hand, Edkawy cultivar showed the highest roots number obtained by IAA at 2.0 mg/L followed by IAA at 3mg/L.Whereas, IAA at1.5 mg/L, IBA at 2.0 and 3.0 mg/L showed no growth of roots (Table3) (Figure 2).

Khalili cultivar illustrated that the highest significant root length was clear with MS

Table (2): Effect of medium type and different cytokinin concentrations on number and length (cm) of proliferated shoots/ explant of Khalili and Edkawy grapes during proliferation stage.

Parameter / Treatment	Survival%		Shoot no.		Shoot length(cm)	
	Khalili	Edkawy	Khalili	Edkawy	Khalili	Edkawy
1-¾ MS +2% sucrose +1 mg/ LBAP +1 mg/ L IAA	100 A	100 A	4.0 H	6.0 C	2.5 E	0.5 IJ
2- full MS +2% sucrose +1 mg/ LBAP +1 mg/ L IAA	100 A	100 A	2.6 K	4.0 H	3.1 D	1.1 H
3-¾ MS +2% sucrose +2mg/ LBAP + 0.3 mg/ L IAA	100 A	100 A	3.3 I	5.0 F	0.63 IJ	2.3 EF
5-¾ MS +2% sucrose + 2mg/ LBAP + 0.5 mg/ L IAA	100 A	100 A	5.3 E	4.0 H	0.66 I	3.0 D
6-¾ MS +2% sucrose + 3mg/ LBAP + 0.3 mg/ L IAA	100 A	100A	4.0 H	4.6 G	0.56 IJ	2.1 F
7-1/2 MS +2% sucrose +1 mg/ LBAP +0.5 mg/ L IAA	100 A	100 A	3.0 J	1.0 M	0.6 IJ	10.3 A
8-1/2 MS +2% sucrose + 5µM TDZ	100 A	100 A	2.0 L	2.6 K	0.4 IK	0.23 K
11-3/4 MS +2% sucrose +1 mg/ L BAP +0.5 mg/ L IAA (Khaliliy)	100 A	100 A	9.3 A	5.3 E	4.6 B	3.0 D
12-1/2MS +2% sucrose +0.5 mg/ LBAP +0.1 mg/ L IAA (Edkawy)	100 A	100 A	5.6 D	8.3 B	1.6 G	4.3 D

supplemented with IAA at 3 mg/L (25.8). Significant differences were noticed among IBA levels and IAA, meanwhile the lowest root length was obtained with IAA at 2.0 mg/L and IBA at 3 mg/L .Root length of Edkawy cultivar demonstrated that the high root length was achieved with MS supplemented with 2.0 mg/L IAA followed by 3 mg/L IAA. Table (3) There is an insignificant difference among IAA at 1.5 mg/L, IBA at 2 and 3mg/L. The obtained results are in the same line of Minal et al. (2000) who used IAA for rooting formation of three grapes varieties (Thompson seedless, Sonaka and Tas-e- Ganesh) .Also, Nalwade and Shitole (2004) who stated that using 5.7and 4.92 µM IAA cause rooting induction of 93 and 99 %, respectively (Figure 1).

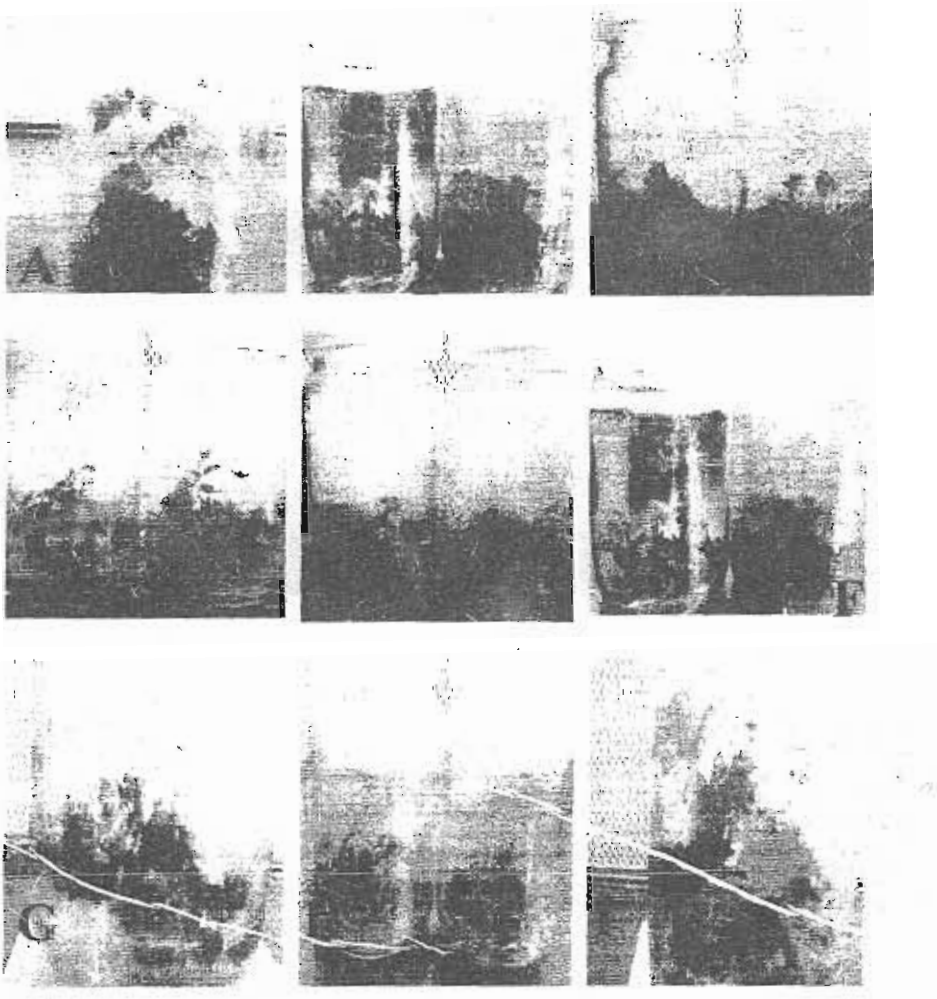
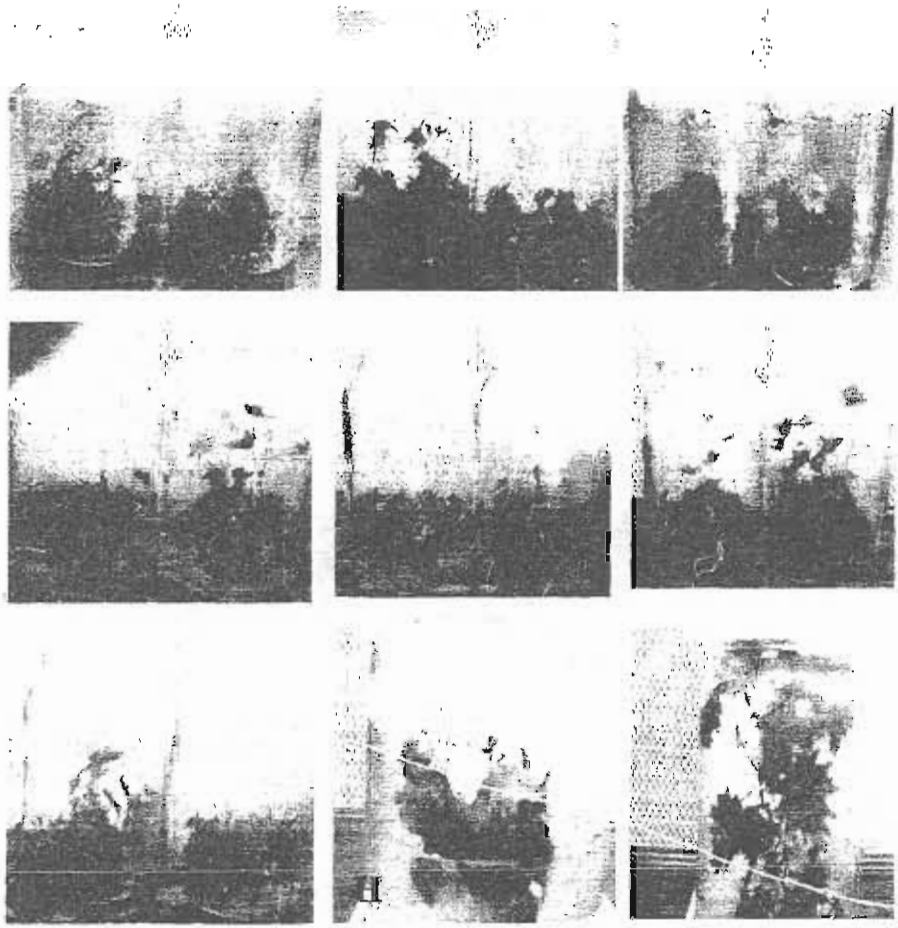


Figure (1): Effect of medium type and different cytokinin concentrations on number and length (cm) of proliferated shoots/ explant of Khalili grape during proliferation stage.

A. 3/4 MS +2% sucrose +1 mg/ L BAP +0.5 mg/ L IAA	**F.** ¾ MS +2% sucrose +2mg/ LBAP + 0.3 mg/ L IAA
B. 1/2MS +2% sucrose +0.5 mg/ LBAP +0.1 mg/ L IAA	**G.** 1/2 MS +2% sucrose +1 mg/ LBAP +0.5 mg/ L IAA
C. ¾ MS +2% sucrose + 2mg/ LBAP + 0.5 mg/ L IAA	**H.** 1/2 MS +2% sucrose + 5µM TDZ
D. ¾ MS +2% sucrose +1 mg/ LBAP +1 mg/ L IAA	**I.** 1/2 MS +2% sucrose + 5µM TDZ
E. ¾ MS +2% sucrose + 3mg/ LBAP + 0.3 mg/ L IAA	



Figure(1)Cont.: Effect of medium type and different cytokinin concentrations on number and length (cm) of proliferated shoots/ explant of Edkawy grape during proliferation stage.

A. 3/4 MS +2% sucrose +1 mg/ L BAP +0.5 mg/ L IAA	**F.** full MS +2% sucrose +1 mg/ LBAP +1 mg/ L IAA
B. ¾ MS +2% sucrose +1 mg/ LBAP +1 mg/ L IAA	**G.** -¾ MS +2% sucrose + 2mg/ LBAP + 0.5 mg/ L IAA
C. 3/4 MS +2% sucrose +1 mg/ L BAP +0.5 mg/ L IAA	**H.** full MS +2% sucrose +1 mg/ LBAP +1 mg/ L IAA
D. -¾ MS +2% sucrose +2mg/ LBAP + 0.3 mg/ L IAA	**I.** 1/2 MS +2% sucrose +1 mg/ LBAP +0.5 mg/ L IAA
E. ¾ MS +2% sucrose + 3mg/ LBAP + 0.3 mg/ L IAA	

Table (3): Effect of different auxins concentrations and combinations added to half strength MS medium on rooting %, number and length (cm) of roots of Khalili and Edkawy grapes during rooting stage.

Parameter / Treatment	Root length (cm)		Root no.		Rooting%)	
	Khalily	Edkawy	Khalily	Edkawy	Khalily	Edkawy
1- MS+2 mg/ L IAA + 0.5 mg / L BAP	12.33 E	34.0 A	2.0 E	9.0 A	33.0 B	100 A
7- MS+3mg/ L IAA +0.5 mg / L BAP	25.8 B	18.0 C	8.0 B	6.0 C	100 A	100 A
8- MS+1.5 mg/ L IAA +0.5 mg / L BAP	18.33 C	0.0 F	3.3 D	0.0 G	90 B	0.0 C
9-1/2 MS+2 mg/ L IBA +A.C.	15.0 D	0.0 F	3.0 D	0.0 G	90 B	0.0 C
10- 1/2 MS+3 mg/ L IBA +A.C.	10.5 E	0.0 F	1.3 F	0.0 G	90 B	0.0 C

Acclimatization stage:

Survival percentage of Khalili and Edkawy plantlets after acclimatization for 8 weeks after rooting were presented in table (4), it was clear that all in vitro rooted shoots of acclimatization in greenhouse where survival percentage was 90% for Khalili and 65% for Edkawy at Vermiculate and Peat moss 1:1 while it was 70% and 50% respectively, at Vermiculate only. These results were contrast with those of Dzazio et al. (2002) who obtained high survival rates during acclimatization of 420-A grape rootstock in Vermiculite (95.8 %).

Table (4): Survival percentage of Khalili and Edkawy grapes cultivars plantlets after acclimatization for 8 weeks.

Cultivar	Survival%	
	Vermiculate and Peat moss 1:1	Vermiculate
Khalili	90 A	70 B
Edkawy	65 C	50 C

A
Edkawy cultivar4

Edkawy cultivar 7

C
Khalily cultivar 4

D
Khalily cultivar 7

E
Khalily cultivar 8

F
Khalily cultivar 9

G
Khalily cultivar 10

Figure(2): Effect of different auxins concentrations and combinations added to half strength MS medium on rooting %, number and length (cm) of roots of Khalili and Edkawy grapes during rooting stage.

A. MS+2 mg/ L IAA + 0.5 mg / L BAP	**E.** MS+1.5 mg/ L IAA +0.5 mg / L BAP
B. MS+3mg/ L IAA +0.5 mg / L BAP	**F.** 1/2 MS+2 mg/ L IBA +A.C.
C. MS+2 mg/ L IAA + 0.5 mg / L BAP	**G.** 1/2 MS+3 mg/ L IBA +A.C.
D. MS+3mg/ L IAA +0.5 mg / L BAP	

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إدخال أصناف العنب فى مجموعة زراعة الانسجة للبنك القومى والمصادر الوراثية فى مصر

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تهدف الدراسة الحالية لتحديد بروتوكول لاكثار صنفين من أصناف العنب المحلية المصرية (الخليلي و الادكاوى) باستخدام تقنية زراعة الأنسجة كأحد أهداف البنك القومى للجينات والموارد الوراثية بجمهورية مصر العربية و التى تشمل أيضا حفظ هذه الاصناف حيث تم فصل براعم قمية وعقد ساقية من نباتات بعمر 9 اشهر نامية فى الصوب الزراعية لاكثارها فى المعمل. أثناء مرحلة التأسيس كانت أعلى نسبة في عدد الأفرع لمنفصلات العقد الساقية وأيضا عدد الأوراق تم الحصول عليها باستخدام بيئة موراشيج و سكوج نصف تركيز (1962) والتى تحتوى على إندول أسيتيك أسيد بتركيز 2.0 مللجرام/ لتر و 2% سكروز.

فى مرحلة التضاعف أستخدم 4 تركيزات من بنزيل أمينو بيورين بتركيز(0.5,1.0,2.0 and 3.0 mg/L وتركيز واحد من TDZ (0.05 mg/L)، 3 تركيزات من بيئة موراشيج وسكوج (كاملة ، 3/4 ، 1/2) .وقد بينت النتائج أن أعلى معدل للتضاعف تم الحصول عليه لصنف الخليلى باستخدام بيئة موراشيج و سكوج بتركيز 4/3 قوة تحتوى على بنزيل أمينو بيورين بتركيز 1.0 مللجرام/ لتر بالإضافة الى إندول أسيتيك أسيد بتركيز 0.05 مللجرام /لتر اما بالنسبة لصنف الادكاوى كان أعلى معدل للتضاعف تم الحصول علية باستخدام بيئة موراشيج و سكوج بتركيز 1/2 قوة تحتوى على بنزيل أمينو بيورين بتركيز 0.5 مللجرام/ لتر بالاضافة الى إندول أسيتيك أسيد بتركيز 0.1 مللجرام /لتر.

بالنسبة لمرحلة التجذير ففى صنف الخليلى كانت أعلى نسبة تجذير (100 %) وأعلى طول جذور وأعلى عدد جذور تم الحصول عليها فى بيئة موراشيج وسكوج كاملة التركيز المحتوية على بنزيل أمينو بيورين بتركيز 0.5 مللجرام/ لتر بالاضافة الى إندول أسيتيك أسيد بتركيز 2.0 مللجرام /لتر. بالنسبة لصنف الادكاوى كانت أعلى نسبة تجذير(100%) وأعلى طول جذور وأعلى عدد جذور تم الحصول عليها فى بيئة موراشيج وسكوج كاملة التركيز المحتوية على بنزيل أمينو بيورين بتركيز 0.5 مللجرام/ لتر بالاضافة الى أندول أسيتيك أسيد بتركيز 3.0 مللجرام /لتر.

تم بنجاح أقلمة نبيتات كل من صنفى العنب (الخليلي و الادكاوى) وكانت نسبة البقاء 90% ، 65% على التوالى بعد 8 أسا بيع من النقل لصوانى الزراعة التى تحتوى على بيت موس و فرمكوليت بنسبة 1:1.