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MICROPROPAGATION AND SALT TOLERANCE EVALUATION IN SOME GRAPE CULTIVARS
I- MICROPROPAGATION

El-Sabrout, M.B.
Pomology Department, Faculty of Agriculture, Alexandria University, Alexandria, Egypt.

ABSTRACT

The present investigation was carried out during three successive years (2000- 2002), in order to study the effect of Murashige and Skoog (1962) medium (MS) supplemented with various concentrations of plant growth regulators on *in vitro* propagation from shoot tip explants of three grape cultivars namely, Early Superior, Flame seedless and Thompson seedless.

The main results of the three tested grape cvs. can be summarized in the following points:

1- The MS medium containing 1.0 $mg1^{-1}$ BA + 0.01 $mg1^{-1}$ NAA produced the highest shoot proliferation percentage, average number of proliferated shoots per shoot tip explant and average length of proliferated shoot from shoot tip cultures.
2- The MS medium containing 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin produced the highest shoot multiplication percentage, average number of new proliferated shoots per original shoot and average length of new proliferated shoot from shoot cultures.
3- The 1/2 MS medium containing 0.2 $mg1^{-1}$ IBA produced the highest rooting percentage, average number of roots per shoot and average root length per shoot in shoot cultures.
4- Flame seedless cv. was the super to produce the highest shoot proliferation from shoot tip explant, shoot multiplication from original shoot and rhizogenesis values, followed by Early Superior cv., while Thompson seedless one was the lowest in that respect.

INTRODUCTION

The grapes are considered the first economic crop in the world and the second in Egypt. Their cultivated area in Egypt comes second after *citrus* and attained 148406 feddan producing about 1078912 ton of fruits according to the statistics of the Ministry of Agriculture and Land Reclamation, Cairo, 2001.

New grape cultivars have been introduced to Egypt such as Flame seedless and Early Superior. It is important to study the possibility of cultivating them under the Egyptian environment, also, to evaluate these cultivars under different stress conditions. This might help to spread out their cultivation area to include the new reclaimed lands.

In vitro propagation of grapevines through shoot tip culture and enhanced axillary bud breaking has been used by Meyerson *et al.*, (1995) who reported that plant micropropagation offers rapid clonal production of desirable grape cultivars.

The present study dealt with *in vitro* propagation of three grape cultivars Early Superior, Flame seedless and Thompson seedless using shoot tip cultures.

MATERIALS AND METHODS

The present investigation was carried out during three successive years (2000- 2002), in order to study the possibility of using tissue culture technique for rapid and economical *in vitro* propagation of three grape cultivars namely, Early Superior, Flame seedless and Thompson seedless (*Vitis vinifera* L.). Factors considered were the determination of the optimum combination and concentration of plant growth regulators, which are responsible to obtain the good shoot proliferation, multiplication and rooting.

1. Shoot Proliferation

In the present investigation, the effect of benzyladenine (BA) and naphthalene acetic acid (NAA) combinations on shoot proliferation from shoot tip explants of the tested grape cultivars was studied.

1.1. Plant Material

Terminal parts (3-4 cm in length) from newly growing vigorously shoots (10 cm long) were cut out from one – year – old plants of grape cultivars under study, which are grown in a greenhouse at the Agricultural Experiment Station of Alexandria University. The plant material was collected early in the growing season (mid – April) of each year.

1.2. Culture Media

The culture media tested for *in vitro* shoot proliferation were consisted of the basic salts and vitamins of Murashige and Skoog (1962) culture medium (MS) at full strength supplemented with sucrose at the rate of 30 $g1^{-1}$. Difco-Bacto agar was added at the rate of $8g1^{-1}$. Plant growth regulators included benzyladenine (BA) at 0.0, 0.5, 1.0 and 2.0 $mg1^{-1}$ and α-naphthalene acetic acid (NAA) at 0.000, 0.005, 0.010 and 0.020 $mg1^{-1}$ were supplemented solely or in various combinations and concentrations in MS media as indicated in Tables (1 – 3) and took the code C_1 to C_{16}. The pH of shoot proliferation media was adjusted to 5.8 using NaOH and HCl before adding agar. The culture tubes were covered with cotton and capped with aluminum foil. The media were autoclaved at 121°C for 20 min., then left to cool and harden for 24 hrs. before being used.

1.3. Sterilization

The plant material were washed with running tap water for 30 minutes. Shoot tip explants (1-2 cm) were excised from the shoot terminal parts using a scalpel blade and a forceps, in a sterile Petridish, under aseptic conditions.

Shoot tip explants were surface sterilized in a laminar flow hood by immersing them in 70% ethanol solution for 3 min., followed by dipping them in 10% sodium hypochloride (Na O Cl) for 10 min., then rinsing three times in sterile redistilled water, and transferred to culture tubes.

1.4. Culture Procedures

Shoot tip explants were cultured in glass culture tubes (180 x 25 mm) containing 15 ml (each) of shoot proliferation media. One explant per each culture tube. After 4 weeks from culturing date, the shoot proliferation percentage, average number of shoots produced per explant and average

length (cm) of proliferated shoots were recorded for shoot tip cultures of the studied grape cultivars.
The shoot proliferation percentage calculated as follows:

$$\text{Shoot proliferation \%} = \frac{\text{Number of shoot tip explants proliferated shoots}}{\text{Total number of shoot tip explants}} \times 100$$

The resultant proliferated shoots were used as a mother stock explants for the subsequent experiments of shoot multiplication.

1.5. Culture Conditions

All the shoot tip cultures were placed in growth culture room under fluorescent light with 16 hrs illumination, followed by 8 hrs dark period, at 24 ± 2°C, for 4 weeks.

1.6. Statistical Analysis

The shoot proliferation experiments were designed as three replicates per each treatment, six shoot tip explants each, in a complete randomized design and the statistical procedures were applied according to Steel and Torrie (1980). The treatments of BA and NAA either solely or in combinations with MS culture medium were represented by 16 ones as indicated in Tables (1 to 3) and took the medium code C_1 to C_{16}.

2. Shoot Multiplication

The shoot multiplication experiments aimed to obtain the best combination and concentration of benzyladenine (BA) and zeatin which allowed high shoot multiplication percentage, average number and length (cm) of uniform new proliferated shoots to be used through the subsequent experiments of rooting.

2.1. Plant Material

The original proliferated shoots (longer than 1 cm) that were produced from the shoot tip cultures (the former experiments) were carefully excised under aseptic conditions, transferred individually to culture glass jars containing the shoot multiplication media and left for 4 weeks.

2.2. Culture Media

The Murashige and Skoog (1962) (MS) was used at full strength for the shoot multiplication experiments. The culture media were supplemented with sucrose at $30g1^{-1}$ + agar at $7\ g1^{-1}$. Plant growth regulators included benzyladenine (BA) at 0.0, 0.5, 1.0 and 2.0 $mg1^{-1}$ and zeatin at the same concentrations mentioned before, were supplemented solely or in various combinations and concentrations in MS culture media. The pH of the shoot multiplication media was adjusted to 5.8 using NaOH and HCl before adding agar. The culture jars (120 x 70 mm) filled with 50 ml (each) of shoot multiplication media. The culture media autoclaved at 121°C for 20 min., then left to cool and harden for 24 hrs before being used.

2.3. *Culture Procedures*

The original proliferated shoots were cultured vertically on each cytokinin- combination. One original shoot explant cultured in each culture jar.

The shoot multiplication percentage, average number of new proliferated shoots, produced from each original shoot explant, and average length of new proliferated shoots (cm) were recorded after 4 weeks of original shoot culture. The resultant proliferated shoots were used as a mother cultures for the subsequent rooting experiments.
The shoot multiplication percentage calculated as follows:

$$\text{Shoot multiplication \%} = \frac{\text{No. of cultured jars with multiple shoots}}{\text{Total no. of cultured jars}} \times 100$$

2.4 Culture Conditions

All the shoot cultures were grown at 24 ± 2°C, in a 16/8 hrs light/dark cycle with fluorescent light, for 4 weeks.

2.5 Statistical Analysis

In the shoot multiplication experiments, each treatment consisted of three replicates with six original shoots for each in a complete randomized design and the statistical procedures were applied according to Steel and Torrie (1980). The treatments of BA and zeatin either solely or in combinations with MS culture medium were represented by 16 ones as indicated in Tables (4 to 6) and took the medium code from C_1 to C_{16}.

3. Rooting of Mult0iplicated Shoots (Rhizogenesis)

These experiments aimed to examine the effect of two auxins [α-naphthalene acetic acid (NAA) and indole – 3 – butyric acid (IBA)] either solely at different concentrations on rooting of multiplicated shoots.

3.1. Plant Material

The rooting experiments were carried out on uniformity and vigorously growing proliferated shoots (≥ 2 cm in length) derived from the shoot multiplication experiments which mentioned previously.

3.2. Culture Media

The Murashige and Skoog (1962) medium (MS) at half strength (1/2 MS) was used for rooting media. Naphthalene acetic acid (NAA) and indole – 3 – butyric acid (IBA) at 0.0, 0.1, 0.2, 0.3 and 0.4 $mg1^{-1}$ were added solely in 1/2 MS rooting media. These media were supplemented with sucrose $20g1^{-1}$. Solidification of the rooting media was achieved by 6 $g1^{-1}$ agar. The pH of these media was adjusted to 5.7 before adding agar. The media were dispensed in glass culture tubes (180 × 25 mm) with 15 ml (each), plugged with cotton and covered with aluminum foil. The rooting media autoclaved at 121°C for 20 min. then left to cool and harden for 24 hrs before being used.

3.3. Culture Procedures

Proliferated shoots were carefully excised under aseptic conditions from actively multiplying cultures and inserted vertically into culture tubes containing rooting media. One proliferated shoot cultured in a culture tube.
The rooting percentage, average number of roots per shoot and average root length (cm) of shoot cultures of the three grape cultivars were recorded after 4 weeks of culturing date.

The rooting percentage calculated as follows:

$$\text{Rooting \%} = \frac{\text{No. of cultured tubes with rooted shoots}}{\text{Total no. of cultured tubes}} \times 100$$

3.4. Culture Conditions

The shoot cultures were incubated on racks in growth culture room at a temperature of 24 ± 2 °C, with 16 hrs photoperiod provided by white fluorescent light, followed by 8 hrs dark period, for 4 weeks.

3.5. Statistical Analysis

In rooting experiments, each treatment consisted of three replicates with six shoots each in a completely randomized design and the statistical procedures were applied according to Steel and Torrie (1980). The different auxin concentrations in 1/2 MS rooting media were represented by 9 treatments as indicated in Tables (7 to 9) and took the treatment code from T_1 to T_9.

All the experiments were repeated for three years.

RESULTS AND DISCUSSION

1. Shoot Proliferation

1.1. Early Superior Cultivar

The data of Table (1) indicated that, the percentage of shoot proliferation from shoot tip explant was significantly the highest (88.89%) on MS medium combined with 1.0 $mg1^{-1}$ BA + 0.01 $mg1^{-1}$ NAA (C_{11}). On the contrary, the lowest percentage (5.56%) was significantly resulted in MS medium without the addition of growth regulators (C_1).

Table (1): Effect of BA and NAA solely or in combinations on shoot proliferation percentage, average number of proliferated shoots per shoot tip explant and average length of proliferated shoot in grape cv. "Early Superior" cultures.

Medium code	Growth regulators in $mg1^{-1}$		Shoot proliferation[x] (%)	Av. no. of proliferated shoots/explant	Av. length of proliferated shoot (cm)
	BA	NAA			
C_1	0.0	0.000	5.56 P*	1.00 O	0.50 L
C_2	0.5	0.000	38.89 J	3.43 I J	1.52 G H I
C_3	1.0	0.000	33.33 K	3.33 J	1.45 H I
C_4	2.0	0.000	27.78 L	3.00 K	1.40 I
C_5	0.0	0.005	11.11 O	1.50 N	0.60 K L
C_6	0.5	0.005	77.78 C	5.00 C	2.13 C
C_7	1.0	0.005	72.22 D	4.69 D	2.00 D
C_8	2.0	0.005	66.67 E	4.50 E	1.88 D
C_9	0.0	0.010	16.67 N	2.00 M	0.70 K
C_{10}	0.5	0.010	83.33 B	5.33 B	2.31 B
C_{11}	1.0	0.010	88.89 A	5.75 A	2.58 A
C_{12}	2.0	0.010	61.11 F	4.36 F	1.72 E
C_{13}	0.0	0.020	22.22 M	2.50 L	1.00 J
C_{14}	0.5	0.020	55.56 G	4.20 G	1.68 E F
C_{15}	1.0	0.020	50.00 H	3.78 H	1.63 E F G
C_{16}	2.0	0.020	44.44 I	3.50 I	1.56 F G H
L.S.D. 0.05			0.417	0.128	0.123

[x]Values refer to the percentage of shoot tip explants that produced proliferate shoots, after 4 weeks of culture.

*Values followed by the same letters in the same column significantly are not differed at the 0.05 level of probability.

The same Table revealed that, average number of proliferated shoots per shoot tip explant was significantly the highest (5.75) on MS medium combined with 1.0 $mg1^{-1}$ BA + 0.01 $mg1^{-1}$ NAA (C_{11}). In contrast, the lowest average number (1.00) was significantly achieved in MS medium without the addition of growth regulators (C_1).

Results also showed that, average length of proliferated shoot was significantly the highest (2.58 cm) on the same C_{11} medium, whereas, the lowest average length (0.50 cm) was also recorded for C_1 medium.

1.2. Flame Seedless Cultivar

Table (2) showed that, the percentage of shoot proliferation from shoot tip explant was significantly the highest (100%) on MS medium combined with 1.0 $mg1^{-1}$ BA + 0.01 $mg1^{-1}$ NAA (C_{11}). In contrast, the lowest percentage (11.11%) was significantly resulted in MS medium without the addition of growth regulators (C_1).

Results in Table (2) indicated that, average number of proliferated shoots per explant was significantly the highest (6.39) on MS medium combined with 1.0 $mg1^{-1}$ BA + 0.01 $mg1^{-1}$ NAA (C_{11}). On the contrary, the lowest average number (1.00) was significantly recorded in MS medium without the addition of growth regulators (C_1).

The same Table also revealed that, average length of proliferated shoot was significantly the highest (2.86 cm) on the same C_{11} medium, whereas, the lowest average length (0.50 cm) was significantly achieved in C_1 medium.

Table (2): Effect of BA and NAA solely or in combinations on shoot proliferation percentage, average number of proliferated shoots per shoot tip explant and average length of proliferated shoot in grape cv. "Flame seedless" cultures.

Medium code	Growth regulators in $mg1^{-1}$		Shoot proliferation[x] (%)	Av. no. of proliferated shoots/explant	Av. length of proliferated shoot (cm)
	BA	NAA			
C_1	0.0	0.000	11.11 P*	1.00 O	0.50 N
C_2	0.5	0.000	50.00 J	4.22 J	1.78 H
C_3	1.0	0.000	44.44 K	3.63 K	1.72 I
C_4	2.0	0.000	38.89 L	3. 57 K	1.65 J
C_5	0.0	0.005	16.67 O	2.00 N	0.70 M
C_6	0.5	0.005	88.89 C	5.81 C	2.45 C
C_7	1.0	0.005	83.33 D	5.67 D	2.40 C
C_8	2.0	0.005	77.78 E	5.50 E	2.27 D
C_9	0.0	0.010	22.22 N	2.25 M	0.90 L
C_{10}	0.5	0.010	94.44 B	6.00 B	2.62 B
C_{11}	1.0	0.010	100.00 A	6.39 A	2.86 A
C_{12}	2.0	0.010	72.22 F	5.31 F	2.15 E
C_{13}	0.0	0.020	27.78 M	2.60 L	1.23 K
C_{14}	0.5	0.020	66.67 G	5.17 G	2.00 F
C_{15}	1.0	0.020	61.11 H	5.00 H	1.92 G
C_{16}	2.0	0.020	55.56 I	4.60 I	1.87 G
L.S.D.	0.05		0.417	0.104	0.054

[x]**Values refer to the percentage of shoot tip explants that produced proliferate shoots, after 4 weeks of culture.**

*Values followed by the same letters in the same column significantly are not differed at the 0.05 level of probability.

1.3. Thompson Seedless Cultivar

Table (3) indicated that, the percentage of shoot proliferation from shoot tip explant was significantly the highest (83.33%) on MS medium combined with 1.0 $mg1^{-1}$ BA + 0.01 $mg1^{-1}$ NAA (C_{11}). On the contrary, the lowest percentage (5.56%) was resulted in MS medium either containing 0.0 $mg1^{-1}$ BA + 0.005 $mg1^{-1}$ NAA (C_5) or without the addition of growth regulators (C_1).

The same Table showed that, average number of proliferated shoots per explant was significantly the highest (5.07) on MS medium combined with 1.0 $mg1^{-1}$ BA + 0.01 $mg1^{-1}$ NAA (C_{11}). In contrast, the lowest average number (1.00) was noticed in either MS medium containing 0.0 $mg1^{-1}$ BA + 0.005 $mg1^{-1}$ NAA (C_5) or without the addition of growth regulators (C_1).

Results in Table (3) also revealed that, average length of proliferated shoot was significantly the highest (2.00 cm) on the same C_{11} medium, whereas, the lowest average length (0.40 cm) was significantly achieved in C_1 medium.

Table (3): Effect of BA and NAA solely or in combinations on shoot proliferation percentage, average number of proliferated shoots per shoot tip explant and average length of proliferated shoot in grape cv. "Thompson seedless" cultures.

Medium code	Growth regulators in $mg1^{-1}$		Shoot proliferation[x] (%)	Av. no. of proliferated shoots/ explant	Av. length of proliferated shoot (cm)
	BA	NAA			
C_1	0.0	0.000	5.56 O*	1.00 M	0.40 N
C_2	0.5	0.000	33.33 J	3.17 H	1.27 HI
C_3	1.0	0.000	27.78 K	2.80 I	1.20 IJ
C_4	2.0	0.000	22.22 L	2.50 J	1.16 J
C_5	0.0	0.005	5.56 O	1.00 M	0.50 M
C_6	0.5	0.005	72.22 C	4.31 C	1.73 C
C_7	1.0	0.005	66.67 D	4.17 CD	1.65 CD
C_8	2.0	0.005	61.11 E	4.09 DE	1.60 D
C_9	0.0	0.010	11.11 N	1.50 L	0.60 L
C_{10}	0.5	0.010	77.78 B	4.86 B	1.86 B
C_{11}	1.0	0.010	83.33 A	5.07 A	2.00 A
C_{12}	2.0	0.010	55.56 F	4.00 E	1.51 E
C_{13}	0.0	0.020	16.67 M	2.00 K	0.80 K
C_{14}	0.5	0.020	50.00 G	3.78 F	1.43 E F
C_{15}	1.0	0.020	44.44 H	3.50 G	1.38 F G
C_{16}	2.0	0.020	38.89 I	3.29 H	1.34 G H
L.S.D. 0.05			0.417	0.152	0.088

[x]Values refer to the percentage of shoot tip explants that produced proliferate shoots, after 4 weeks of culture.

*Values followed by the same letters in the same column significantly are not differed at the 0.05 level of probability.

Regarding the forecited results in Tables (1 to 3), it was cleared that, Flame seedless cultivar produced the highest shoot proliferation percentage (100%), average number of proliferated shoots per shoot tip explant (6.39) and average length of proliferated shoot (2.86 cm), followed by Early

Superior cultivar which tabulated 88.89%, 5.75 and 2.58 cm, respectively. Thompson seedless was the lowest in that respect with shoot proliferation percentage of 83.33%, average number of proliferated shoots per shoot tip explant of 5.07 and average length of proliferated shoot of 2.00 cm.

The same Tables also revealed that, among the tested shoot proliferation media, the MS one combined with 1.0 $mg1^{-1}$ BA + 0.01 $mg1^{-1}$ NAA (C_{11}) was the best medium to produce the highest values for shoot proliferation percentage, average number of proliferated shoots per explant and average length of proliferated shoot from cultures of the three tested grape cultivars. Benzyladenine at 1.0 $mg1^{-1}$ and NAA at 0.01 $mg1^{-1}$ were the most effective combination for shoot proliferation.

The obtained results were in complete agreement with those reported by Tapia and Read (1998). Who found that, the variation in shoot proliferation responses was highly genotype-dependent. Valiant grape hybrid was the most responsive and Vincent grape hybrid was the least. They also mentioned that, MS + BA at 1.0 $mg1^{-1}$ + NAA at 0.01 $mg1^{-1}$ proved to be the best combination for shoot proliferation of axillary bud explants. The same authors also, stated that NAA added to the medium had a positive effect on *in vitro* shoot proliferation of all grape cultivars studied. Also with Yae *et al.*, (1990) who found that, shoot proliferation from shoot tip explant of grape cv. Rizamat was the highest on the combination medium of MS + 1.0 $mg1^{-1}$ BA + 0.05 $mg1^{-1}$ NAA .

The present results partially agreed with those obtained by Cheng and Reisch (1989) with grapevines. They mentioned that the best shoot proliferation from axillary bud explants was resulted in MS medium + 4 µM BA. In the meantime, Cholvadova (1989) reported that the best shoot proliferation from meristems (apical and axial buds) of grapevines accomplished in MS medium + 5 to 7 µM BA. Also, Gray and Klein (1989) with grapevines reported that C_2D medium (a modification of MS) + 20 µM BA produced significantly more shoot/apex (4.0) in shoot cultures.

In addition, Hartl and Jelaska (1989) reported that the best shoot proliferation from apical meristem of grapevine noticed on basal MS medium containing 8.9 µ M BA. Furthermore, Martinez *et al.*, (1989) reported that the best shoot proliferation from microcuttings of grapevine was obtained on MS medium containing BA at 1.0 mgl^{-1} + GA_3 at 0.5 mgl^{-1}. Moreover, Lee and Wetzstein (1990) reported that best total shoot production from nodal segments of Muscadine grape was obtained on MS basal medium supplemented with 10 µ M BA. Meanwhile, Blazina *et al.*, (1991) found that the best meristem proliferation from nodal segments of "Zelen" grape cultivar was resulted in MS medium + 5 µM BAP + 1.1 µM IAA.

In this concept, Gray and Benton (1991 a and b) reported that the optimum rate of shoot proliferation from shoot apical meristems of vineyard – grown vines (Fry, Carlos and Dixie grape cultivars) was recorded in full – strength MS medium + 5 to 20 µM BA. The explants cultured on this medium produced the highest average number of shoots/ apex (3.4 to 3.8). Also, Dalal *et at.*, (1993) found that the best shoot proliferation from shoot tip

explants resulted in MS medium + 10 μM BA. In the same line, Peixoto and Pasqual (1994) reported that the best shoot proliferation from stem segments of grapevine was obtained on C_2D medium (a modification of MS) + BA at 1.0 mgl^{-1}. Al – Maarri and Al- Ghamdi (1995) reported that the highest shoot proliferation from shoot tip explants of grape cultivars Banaty and Khalas achieved on MS + 0.2 $mg1^{-1}$ NAA + 0.4 $mg1^{-1}$ GA_3 + 1.0 $mg1^{-1}$ BA.

In the meantime, Compton and Gray (1995) mentioned that the optimum shoot proliferation (88%) from shoot apices of "Southern Home" hybrid grape resulted in MS medium + 5 μM BA. Shoot tip explant cultured in this medium produced an average of 3 axillary shoots every 5 weeks. In addition, Meyerson *et al.*, (1995) reported that shoot micropropagation from shoot tip explants of grape cultivars accomplished in C_2D medium (a modification of MS) + 5 μM BA. The best response of shoot production was found in Dog Ridge cultivar with 5.8 shoots per apex and the worst in Dixie cultivar with 2.2 shoots per apex. Furthermore, Peixoto and Pasqual (1995) stated that the nutrient media for shoot micropropagation of grapevine rootstock RR-101-14 consisted of C_2D culture medium + BA at 0.5 mgl^{-1}. In the same line, Torregrosa and Bouquet (1995) found that the best shoot micropropagation (the highest number of shoots/ explant) from axillary budding of 6 grapevine hybrids recorded in MS/ 2 + BA at 4.4 μM.

Recently, Biasi *et al.*, (1998) reported that the best shoot proliferation of Jales grapevine rootstock noticed in MS + 10 μ M BA. In addition, Shahin (1997) and Wafaa *et al.*, (1999) reported that MS medium + 0.2 mgl^{-1} BA gave the best results in terms of uniform normal shoots proliferated from stem node cultures of Flame seedless grape cultivar.

On the other hand, the above mentioned results disagreed with those reported by Peixoto and Pasqual (1994). They found that NAA had an adverse effect on shoot proliferation from stem segments of grapevine rootstock RR-101-14.

2. Shoot Multiplication

2.1. Early Superior Cultivar

The results in Table (4) indicated that, the percentage of shoot multiplication from original shoot after one subsequent subculture was significantly the highest (83.33%) on MS medium combined with 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin (C_7). On the contrary, the lowest percentage (5.56%) was achieved in MS medium either containing 0.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin (C_5) or without the addition of cytokinins (C_1).

Such results also revealed that, average number of new proliferated shoots per original shoot was significantly the highest (4.00) on MS medium combined with 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin (C_7). In contrast, the lowest one (1.00) was resulted in MS medium either containing 0.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin (C_5) or without the addition of cytokinins (C_1).

This Table also showed that, average length of new proliferated shoot was significantly the highest (2.55 cm) on the same C_7 medium, whereas, the lowest one (0.60 cm) was significantly noticed in C_1 medium.

Table (4): Effect of BA and zeatin solely or in combinations on shoot multiplication percentage, average number of new proliferated shoots per original shoot and average length of new proliferated shoot in grape cv. "Early Superior" cultures.

Medium code	Cytokinins in $mg1^{-1}$		Shoot multiplication[y] (%)	Av. no. of new proliferated shoots/original shoot	Av. length of new proliferated shoot (cm)
	BA	Zeatin			
C_1	0.0	0.0	5. 56 O*	1.00 K	0.60 N
C_2	0.5	0.0	50.00 G	2.78 F	2.09 F
C_3	1.0	0.0	55.56 F	3.00 E	2.18 E
C_4	2.0	0.0	61.11 E	3.18 D	2.23 E
C_5	0.0	0.5	5.56 O	1.00 K	1.32 M
C_6	0.5	0.5	72.22 C	3.62 B	2.40 C
C_7	1.0	0.5	83.33 A	4.00 A	2.55 A
C_8	2.0	0.5	22.22 L	1.75 J	1.60 J
C_9	0.0	1.0	11.11 N	1.00 K	1.51 K
C_{10}	0.5	1.0	27.78 K	2.00 I	1.74 I
C_{11}	1.0	1.0	66.67 D	3.42 C	2.31 D
C_{12}	2.0	1.0	77.78 B	3.71 B	2.47 B
C_{13}	0.0	2.0	16.67 M	1.67 J	1.43 L
C_{14}	0.5	2.0	33.33 J	2.17 H	1.86 H
C_{15}	1.0	2.0	38.89 I	2.43 G	1.92 H
C_{16}	2.0	2.0	44.44 H	2.63 F	2.00 G
L.S.D. 0.05			0.417	0.162	0.064

[y]Values refer to the percentage of original shoots that produced multiple shoots, after 4 weeks of culture.

*Values followed by the same letters in the same column significantly are not differed at the 0.05 level of probability.

2.2. Flame Seedless Cultivar

The data in Table (5) cleared that, the percentage of shoot multiplication was significantly the highest (94.44 %) on MS medium containing 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin (C_7). In contrast, the lowest percentage (11.11%) was significantly achieved in MS medium without the addition of cytokinins (C_1).

The results revealed that, average number of new proliferated shoots per original shoct was significantly the highest (4.47) on MS medium combined with 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin (C_7). On the contrary, the lowest average number (1.00) was significantly noticed in MS medium without the addition of cytokinins (C_1).

The same data also indicated that, average length of new proliferated shoot was significantly the highest (2.73 cm) on the same C_7 medium, whereas, the lowest one (1.00 cm) was significantly resulted in C_1 medium.

Table (5): Effect of BA and zeatin solely or in combinations on shoot multiplication percentage, average number of new proliferated shoots per original shoot and average length of new proliferated shoot in grape cv. "Flame seedless" cultures.

Medium code	Cytokinins in $mg1^{-1}$		Shoot multiplication[y] (%)	Av. no. of new proliferated shoots/original shoot	Av. length of new proliferated shoot (cm)
	BA	Zeatin			
C_1	0.0	0.0	11.11 P*	1.00 N	1.00 O
C_2	0.5	0.0	61.11 G	3.00 G	2.17 F
C_3	1.0	0.0	66.67 F	3.17 F	2.20 F
C_4	2.0	0.0	72.22 E	3.39 E	2.32 E
C_5	0.0	0.5	16.67 O	1.00 N	1.40 N
C_6	0.5	0.5	83.33 C	3.80 C	2.51 C
C_7	1.0	0.5	94.44 A	4.47 A	2.73 A
C_8	2.0	0.5	33.33 L	2.00 K	1.70 K
C_9	0.0	1.0	27.78 M	1.80 L	1.61 L
C_{10}	0.5	1.0	38.89 K	2.14 K	1.79 J
C_{11}	1.0	1.0	77.78 D	3.57 D	2.40 D
C_{12}	2.0	1.0	88.89 B	4.00 B	2.65 B
C_{13}	0.0	2.0	22.22 N	1.25 M	1.54 M
C_{14}	0.5	2.0	44.44 J	2.38 J	1.85 I
C_{15}	1.0	2.0	50.00 I	2.56 I	1.97 H
C_{16}	2.0	2.0	55.56 H	2.80 H	2.08 G
L.S.D. 0.05			0.417	0.152	0.050

[y]Values refer to the percentage of original shoots that produced multiple shoots, after 4 weeks of culture.

*Values followed by the same letters in the same column significantly are not differed at the 0.05 level of probability.

2.3. Thompson Seedless Cultivar

The results in Table (6) indicated that, the percentage of shoot multiplication was significantly the highest (77.78%) on MS medium combined with 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin (C_7). On the contrary, the lowest percentage (5.56%) was resulted in MS medium either containing 0.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin (C_5) or without the addition of cytokinins (C_1).

Such results also revealed that, average number of new proliferated shoots per original shoot was significantly the highest (3.57) on MS medium combined with 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin (C_7). In contrast, the lowest one (1.00) was achieved in MS medium either containing 0.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin (C_5) or without the addition of cytokinins (C_1).

The same results also showed that, average length of new proliferated shoot was significantly the highest (2.40 cm) on MS medium combined with 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin (C_7), whereas, the lowest one (0.50 cm) was significantly noticed in MS medium without the addition of cytokinins (C_1).

Table (6): Effect of BA and zeatin solely or in combinations on shoot multiplication percentage, average number of new proliferated shoots per original shoot and average length of new proliferated shoot in grape cv. "Thompson seedless" cultures.

Medium code	Cytokinins in $mg1^{-1}$		Shoot multiplication[y] (%)	Av. no. of new proliferated shoots/original shoot	Av. length of new proliferated shoot (cm)
	BA	Zeatin			
C_1	0.0	0.0	5.56 N*	1.00 L	0.50 P
C_2	0.5	0.0	44.44 G	2.38 G	1.91 G
C_3	1.0	0.0	50.00 F	2.56 F	2.00 F
C_4	2.0	0.0	55.56 E	2.80 E	2.06 E
C_5	0.0	0.5	5.56 N	1.00 L	1.24 O
C_6	0.5	0.5	66.67 C	3.17 C	2.21 C
C_7	1.0	0.5	77.78 A	3.57 A	2.40 A
C_8	2.0	0.5	16.67 L	1.33 K	1.51 L
C_9	0.0	1.0	11.11 M	1.00 L	1.43 M
C_{10}	0.5	1.0	22.22 K	1.50 J	1.57 K
C_{11}	1.0	1.0	61.11 D	3.00 D	2.14 D
C_{12}	2.0	1.0	72.22 B	3.39 B	2.33 B
C_{13}	0.0	2.0	5.56 N	1.00 L	1.36 N
C_{14}	0.5	2.0	27.78 J	1.80 I	1.65 J
C_{15}	1.0	2.0	33.33 I	2.00 H	1.73 I
C_{16}	2.0	2.0	38.89 H	2.14 H	1.80 H
L.S.D. 0.05			0.832	0.162	0.047

[y]Values refer to the percentage of original shoots that produced multiple shoots, after 4 weeks of culture.

*Values followed by the same letters in the same column significantly are not differed at the 0.05 level of probability.

According to the results in Tables (4 to 6), it was noticed that, Flame seedless cultivar was the super to produce the highest shoot multiplication percentage (94.44%), average number of new proliferated shoots per original shoot (4.47) and average length of new proliferated shoot (2.73 cm), followed by Early Superior cultivar which tabulated 83.33%, 4.00 and 2.55 cm, respectively. Thompson seedless was the lowest in that respect with shoot multiplication percentage of 77.78% , average number of new proliferated shoots per original shoot of 3.57 and average length of new proliferated shoot of 2.40 cm.

The same Tables also revealed that, among the tested shoot multiplication media, the MS one containing 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin (C_7) was the best medium to produce the highest shoot multiplication percentage, average number of new proliferated shoots per original shoot and average length of new proliferated shoot from shoot cultures of the three tested grape cultivars.

These findings were in complete agreement with those reported by Diaz *et al.*, (1995) who found that the best shoot multiplication of grape cv. "Albarino" accomplished on MS medium + 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ zeatin. They also partially agreed with those obtained by Lee and Wetzstein (1990) who reported that the best shoot multiplication of "Muscadine" grape was obtained on MS medium + 10 µM BA. Moreover, Singh *et al.*, (1992) reported that the maximum rate of shoot multiplication in grape cultivar "Perlette" was noticed in MS medium + 10 µM BA. In the same line, Al –

Maarri and Al-Ghamdi (1995) found that the shoot multiplication rates of up to 3- fold/month in grape cultivars "Banaty" and "Khalas" were obtained on MS medium + 0.2 $mg1^{-1}$ NAA + 0.4 $mg1^{-1}$ GA_3 + 1.0 $mg1^{-1}$ BA. In the meantime, Torregrosa and Lopez (1996) stated that addition of BA to the culture medium stimulated shoot proliferation from axillary buds and increased the shoot multiplication yield. They also found that the best results were obtained when explants of grapevine (*Vitis x Muscadinia* hybrids) were cultured in the presence of 4.4 μM BA.

Recently, Sivritepe and Eris (1998 and 1999) reported that the best shoot multiplication of grape rootstocks and cultivars was obtained on MS medium + 5 μM BA. In this concept, Shahin (1997) and Wafaa *et al.*, (1999) reported that MS medium + 0.2 $mg1^{-1}$ BA gave the best results in terms of uniform normal shoots multiplicated from stem node cultures of Flame seedless grape cultivar.

However, the above mentioned results were inagreement with those obtained by Martinez *et al.*, (1989). They reported that shoot multiplication of grapevine achieved on half-strength MS (growth regulator-free medium). In addition, Biasi *et al.*, (1998) found that shoot multiplication of "Jales" grapevine rootstock can be achieved on half-strength MS medium growth-regulator-free.

3. Rooting (Rhizogenesis)

3.1 Early Superior Cultivar

The results in Table (7) indicated that, the rooting percentage of proliferated shoots was significantly the highest (77.78%) on 1/2 strength MS medium combined with 0.2 $mg1^{-1}$ IBA (T_7). On the contrary, the lowest percentage (11.11%) was significantly resulted in 1/2 MS medium without the addition of auxin (T_1).

Table (7): Effect of NAA and IBA concentrations on rooting percentage of proliferated shoots (derived from original shoot), average number of roots per shoot and average root length in grape cv. "Early Superior" cultures.

Treatment code	Auxins in $mg1^{-1}$		Rooting[z] (%)	Av. no. of roots/ proliferated shoot	Av. root length (cm)
	NAA	IBA			
T_1	0.0	0.0	11.11 I*	1.00 I	1.42 I
T_2	0.1	0.0	61.11 D	5.18 D	2.80 D
T_3	0.2	0.0	66.67 C	5.75 C	2.96 C
T_4	0.3	0.0	50.00 F	3.00 F	2.00 F
T_5	0.4	0.0	38.89 H	1.86 H	1.75 H
T_6	0.0	0.1	72.22 B	7.00 B	3.26 B
T_7	0.0	0.2	77.78 A	8.29 A	4.50 A
T_8	0.0	0.3	55.56 E	4.20 E	2.35 E
T_9	0.0	0.4	44.44 G	2.00 G	1.90 G
L.S.D. 0.05			0.573	0.133	0.064

[z]Values refer to the percentage of proliferated shoots that produced roots, after 4 weeks of culture.

*Values followed by the same letters in the same column significantly are not differed at the 0.05 level of probability.

Such results also revealed that, average number of roots per shoot was significantly the highest (8.29) on 1/2 MS medium containing 0.2 $mg1^{-1}$ IBA (T_7), whereas, the lowest one (1.00) was significantly recorded in 1/2 MS medium without the addition of auxin (T_1).

The same Table showed that, average root length per shoot was significantly the highest (4.50 cm) on 1/2 MS medium containing 0.2 $mg1^{-1}$ IBA (T_7). In contrast, the lowest one (1.42 cm) was significantly noticed in 1/2 MS medium without the addition of auxin (T_1).

3.2 Flame Seedless Cultivar

The data in Table (8) cleared that, the rooting percentage of proliferated shoots was significantly the highest (88.89%) on 1/2 MS medium containing 0.2 $mg1^{-1}$ IBA (T_7). In contrast, the lowest percentage (16.67%) was significantly achieved in 1/2 MS medium without the addition of auxin (T_1).

The results indicated that, average number of roots per shoot was significantly the highest (9.19) on 1/2 MS medium containing 0.2 $mg1^{-1}$ IBA (T_7), whereas, the lowest one (1.33) was significantly resulted in 1/2 MS medium without the addition of auxin (T_1).

The same Table revealed that, average root length per shoot was significantly the highest (5.61 cm) on 1/2 MS medium containing 0.2 $mg1^{-1}$ IBA (T_7). On the contrary, the lowest one (1.80 cm) was significantly noticed in 1/2 MS medium without the addition of auxin (T_1).

Table (8): Effect of NAA and IBA concentrations on rooting percentage of proliferated shoots (derived from original shoot), average number of roots per shoot and average root length in grape cv. "Flame seedless" cultures.

Treatment code	Auxins in $mg1^{-1}$		Rooting[z] (%)	Av. no. of roots/ proliferated shoot	Av. root length (cm)
	NAA	IBA			
T_1	0.0	0.0	16. 67 I[*]	1.33 I	1.80 I
T_2	0.1	0.0	72.22 D	6.08 D	3.04 D
T_3	0.2	0.0	77.78 C	6.86 C	3.80 C
T_4	0.3	0.0	61.11 F	4.18 F	2.75 F
T_5	0.4	0.0	50.00 H	2.22 H	2.40 H
T_6	0.0	0.1	83.33 B	8.00 B	4.22 B
T_7	0.0	0.2	88.89 A	9.19 A	5.61 A
T_8	0.0	0.3	66.67 E	5.25 E	2.90 E
T_9	0.0	0.4	55.56 G	3.20 G	2.63 G
L.S.D. 0.05			0.573	0.065	0.032

[z]Values refer to the percentage of proliferated shoots that produced roots, after 4 weeks of culture.

[*]Values followed by the same letters in the same column significantly are not differed at the 0.05 level of probability.

3.3 Thompson Seedless Cultivar

The results in Table (9) indicated that, the rooting percentage of proliferated shoots was significantly the highest (72.22%) on 1/2 MS medium containing 0.2 $mg1^{-1}$ IBA (T_7). On the contrary, the lowest percentage (5.56%) was significantly resulted in 1/2 MS medium without the addition of auxin (T_1).

The data revealed that, average number of roots per shoot was significantly the highest (6.77) on 1/2 MS medium containing 0.2 $mg1^{-1}$ IBA (T_7). In contrast, the lowest one (1.00) was significantly achieved in 1/2 MS medium without the addition of auxin (T_1)

The same Table cleared that, average root length per shoot was significantly the highest (3.00 cm) on 1/2 MS medium containing 0.2 $mg1^{-1}$ IBA (T_7), whereas, the lowest one (1.20 cm) was significantly recorded in 1/2 MS medium without the addition of auxin (T_1).

Table (9): Effect of NAA and IBA concentrations on rooting percentage of proliferated shoots (derived from original shoot), average number of roots per shoot and average root length in grape cv. "Thompson seedless" cultures.

Treatment code	Auxins in $mg1^{-1}$		Rooting[z] (%)	Av. no. of roots/ proliferated shoot	Av. root length (cm)
	NAA	IBA			
T_1	0.0	0.0	5.56 I*	1.00 I	1.20 F
T_2	0.1	0.0	55.56 D	4.20 D	2.00 C
T_3	0.2	0.0	61.11 C	5.18 C	2.11 C
T_4	0.3	0.0	44.44 F	2.00 F	1.65 E
T_5	0.4	0.0	33.33 H	1.67 H	1.52 E
T_6	0.0	0.1	66.67 B	6.17 B	2.34 B
T_7	0.0	0.2	72.22 A	6.77 A	3.00 A
T_8	0.0	0.3	50.00 E	3.00 E	1.79 D
T_9	0.0	0.4	38.89 G	1.86 G	1.58 E
L.S.D. 0.05			1.144	0.084	0.130

[z]Values refer to the percentage of proliferated shoots that produced roots, after 4 weeks of culture.

*Values followed by the same letters in the same column significantly are not differed at the 0.05 level of probability.

Referring to the obtained results in Tables (7 to 9), it is evident that, Flame seedless cultivar was the super to produce the highest rooting percentage (88.89%), average number of roots per shoot (9.19) and average root length per shoot (5.61 cm), followed by Early Superior cultivar which tabulated 77.78% , 8.29 and 4.50 cm, respectively. The rest cultivar (Thompson seedless) was the lowest in that respect with rooting percentage of 72.22%, average number of roots per shoot of 6.77 and average root length per shoot of 3.00 cm.

These results were in accordance with those obtained by Roubelakis– Angelakis and Zivanovitc (1991). Who reported that a strong genotype – dependent rhizogenic response of grape rootstocks and cultivars to culture medium and auxins was shown.

The same Tables also indicated that, among the tested rooting media, the 1/2 MS one containing 0.2 $mg1^{-1}$ IBA (T_7) was the best medium to produce the highest rooting percentage, average number of roots per shoot and average root length per shoot in shoot cultures of the three tested grape cultivars.

These findings were in complete agreement with those reported by Cholvadova (1989) who found that the most suitable rooting medium in grape

shoot cultures was MS medium with half concentration of mineral salts + 1.0 μM IBA. In addition, Torregrosa and Bouquet (1995) found that MS/2 medium + 1.0 μM IBA stimulated rhizogenesis in 6 grape hybrids.

The obtained results partially agreed with those reported by Lee and Wetzstein (1990). They found that MS medium supplemented with IBA enhanced rooting by increasing rooting percentage and roots number per plantlet of "Muscadine" grape. In the same line, Singh *et al.*, (1992) stated that primary root formation, its growth and branching in grape cultivar "Perlette" was maximum on MS medium supplemented with IBA level of 10 μM. In the meantime, Singh *et al.*, (1993) mentioned that, more than 85% of shoots of Thompson seedless and Perlette cultivars rooted successfully on MS medium containing IBA (1.0 $mg1^{-1}$). Moreover, Al- Maarri and Al-Ghamdi (1995) stated that, greater than 80% rooting of subsequent plantlets of grape cultivars, Banaty and Khalas was achieved on 1/3 – strength MS medium + 0.2 $mg1^{-1}$ IBA. In addition, Compton and Gray (1995) reported that, from 76 to 83% of grape shoots rooted when transferred to MS medium + 1.0 μM NAA or IBA, respectively.

On the other hand, the above mentioned results disagreed with those obtained by Gray and Klein (1989). They reported that rooting of grapevine shoots was obtained on MS medium + 1.0 μM NAA. Moreover, Martinez *et al.*, (1989) reported that rooting of grapevine shoots achieved on MS medium + NAA at 0.01 $mg1^{-1}$. In addition, Stamp *et al.*, (1990) stated that shoots of French colombard and Thompson seedless grape cultivars rooted at a higher frequency on MS medium + 1.0 $mg1^{-1}$ IAA. Furthermore, Blazina *et al.*, (1991) reported that rooting of "Zelen" grape shoots resulted in the half strength MS medium + 1.1 μM IAA.

Recently, Biasi *et al.*, (1998) mentioned that shoots of "Jales" grape rootstock can be achieved in growth regulator - free half – strength MS medium.

Finally, the present study gives a very detailed protocol for *in vitro* propagation of the three tested grape cultivars.

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الإكثار المعملى الدقيق والتقييم لتحمل الملوحة فى بعض أصناف العنب

١- الإكثار المعملى الدقيق

محمد بدر الصبروت
قسم الفاكهة - كلية الزراعة - جامعة الإسكندرية - الإسكندرية - مصر

أجرى هذا البحث خلال ثلاث سنوات متتالية (٢٠٠٠- ٢٠٠٢) بغرض الإكثار المعملى الدقيق لثلاثة أصناف من العنب هى إيرلى سوبير يور وفليم سيدلس والبناتى باستخدام تقنية زراعة الأنسجة وذلك بدراسة تأثير بيئة الزراعة موراشيج وسكوج (١٩٦٢) مضاف إليها تركيزات مختلفة من منظمات النمو النباتية على إنتاج الأفرخ من منفصل القمة النامية وإكثار الأفرخ الناتجة وتجذيرها فى مزارع الأنسجة.
ويمكن تلخيص النتائج الرئيسية لهذه الدراسة فى النقاط التالية:

١- كانت بيئة الزراعة موراشيج وسكوج (١٩٦٢) والمحتوية على ١,٠٠ مليجرام فى اللتر بنزايل أدنين + ٠,٠١ مليجرام فى اللتر نفثالين حمض الخليك أفضل بيئة لإنتاج أعلى قيم لكل من النسبة المئوية لإنتاج الأفرخ ومتوسط عدد الأفرخ الناتجة لكل منفصل للقمة النامية ومتوسط طول الفرخ الناتج من مزارع القمم النامية لأصناف العنب الثلاثة.

٢- كانت بيئة الزراعة موراشيج وسكوج (١٩٦٢) والمحتوية على ١,٠٠ مليجرام فى اللتر بنزايل أدنين + ٠,٥ مليجرام فى اللتر زياتين أفضل بيئة لإنتاج أعلى قيم لكل من النسبة المئوية لإكثار الأفرخ الجديدة من الفرخ الأصلى ومتوسط عدد الأفرخ الجديدة الناتجة من كل فرخ أصلى ومتوسط طول الفرخ الناتج الجديد من مزارع الأفرخ لأصناف العنب الثلاثة.

٣- كانت بيئة الزراعة موراشيج وسكوج (١٩٦٢) عند نصف تركيز أملاحها والمحتوية على ٠,٢ مليجرام فى اللتر إندول حمض البيوتريك أفضل بيئة لإنتاج أعلى قيم لكل من النسبة المئوية للتجذير ومتوسط عدد الجذور لكل فرخ ومتوسط طول الجذر لكل فرخ وذلك فى مزارع الأفرخ لأصناف العنب الثلاثة.

٤- تفوق الصنف فليم سيدلس فى إعطاء أعلى قيم لكل من إنتاج الأفرخ من منفصل القمة النامية وإكثار الأفرخ الجديدة من الفرخ الأصلى وعملية التجذير يليه الصنف إيرلى سوبريور بينما الصنف البناتى فكان أقلهم فى ذلك.