J. Agric. Sci. Mansoura Univ., 28(3): 1969 – 1981, 2003

MICROPROPAGATION OF MALLING MERTON 106 APPLE ROOTSTOCK :
I- SHOOT PROLIFERATION AND MULTIPLICATION

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ABSTRACT

The present research was carried out during three successive years (2000-2002), in order to study the effect of Murashige and Skoog (1962) (MS) culture medium supplemented with various concentrations of plant growth regulators on the shoot proliferation from shoot tip explants and shoot multiplication in Malling Merton 106 (MM106) apple rootstock cultures.

The main results can be summarized in the following points:

1- Shoot proliferation percentage from shoot tip explant, average number of proliferated shoots per shoot tip explant and average length of proliferated shoot were significantly the highest (96.67%, 7.79 and 2.36 cm, respectively) on MS medium + 2.0 $mg1^{-1}$ BA + 0.2 $mg1^{-1}$ NAA.

2- On MS medium + 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin, the percentage of cultures with multiple shoots from original shoot, average number of new proliferated shoots per original shoot and average length of new proliferated shoot were superior, during four successive subcultures.

3- In the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} subcultures, the percentage of cultures with multiple shoots from original shoot was significantly the highest (90.00%, 96.67%, 100.00% and 93.33%, respectively). Average number of new proliferated shoots per original shoot was significantly the highest (7.52, 9.79, 10.00 and 8.21, respectively). Average length of new proliferated shoot was significantly the highest (2.65cm, 2.83cm, 3.00cm and 2.70cm, respectively).

INTRODUCTION

The apples (*Malus domestica* Borkh.) are an important fruit crop in most regions of the world. Apple is considered as one of the important fruit crops in Egypt. The total area devoted for apple cultivation reached 65141 feddan producing about 473588 ton of fruits according to the statistics of the Ministry of Agriculture and Land Reclamation, Cairo, 2001.

Most of the increase on the new established apple orchards was concentrated in Nubaria region (new cultivated area). Nowadays, "Anna" is the most widely apple variety cultivated in Egypt, especially in the new reclaimed land. The suitable rootstock for this cultivar is Malling Merton 106 (semi – dwarfing rootstock). This rootstock is the most recommended apple rootstock in Egypt (Bondok *et al*., 1987).

Apple can be propagated by various means of vegetative propagation such as budding or grafting on rootstocks. The available production of rootstocks that fit the Egyptian environments, through the traditional means of propagation is unsufficient. Moreover, due to some quarantine regulations to prevent introducing of serious pathogens,

importation of rootstocks was lately suspended. Therefore, *in vitro* propagation of apple would be of great importance.

Apple micropropagation has become a commercial reality. Scion and rootstock cultivars of apple can be propagated through tissue culture techniques (James *et al.*, 1988; Predieri and Fasolo, 1989; Ancherani *et al.*, 1990; Druart, 1990 and Korban *et al.*, 1992).

The effects of genotype, explants and culture conditions on apple shoot organogenesis have been studied (Bondok *et al.*, 1987; Fasolo *et al.*, 1989; Dufour, 1990; Theiler – Hedtrich and Theiler – Hedtrich, 1990 and Shawky *et al.*, 1993).

High frequency shoot regeneration from *in vitro* leaves has been succeeded with the clonal apple rootstocks M. 25, M. 26 and M. 27 (James *et al.*, 1984; Jones *et al.*, 1984; Predieri and Fasolo, 1989), otherwise, it was more difficult for M. 9 and MM. 106 (James, 1987).

The first objective of this study was to develop a reliable technique to obtain high shoot proliferation frequency from shoot tip explants of MM 106 apple rootstock. The second objective was to obtain high shoot multiplication frequency from proliferated shoots (derived from shoot tip explants) of MM. 106.

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 Malling Merton 106 (*Malus pumila* Mill.) apple rootstock (MM 106).

The effect of plant growth regulators on the shoot proliferation and shoot multiplication stages for *in vitro* propagation of MM106 rootstock was examined in this study.

1. Shoot Proliferation

The effect of the cytokinin (benzyladenine) to auxin (α- naphthalene acetic acid) ratio in culture media on shoot proliferation from shoot tip explant cultures was studied in this work.

1.1. Plant Material

Shoot tip explants (2mm long) were excised under aseptic conditions, from the new growing terminal shoots. These shoots were collected in March 2000, from actively growing shoots of four-year-old trees grown in a private orchard located at El-Nubaria region, El-Beheira Governorate, Egypt.

1.2. Sterilization

Shoot tip explants were rinsed with running tap water for 10 min., soaked in anti-oxidant solution containing 100 $mg1^{-1}$ ascorbic acid and 150 $mg1^{-1}$ citric acid for 15 min according to Wang *et al.*, (1994). Surface sterilization of the shoot tips was carried out in a laminar flow hood by immersing in 70% ethanol solution for one minute followed by dipping them in

0.2% solution of mercuric chloride ($HgCl_2$) for 3 min., followed by three rinses in sterile distilled water, then transferred to culture tubes.

1.3. Culture Media

The effect of various combinations and concentrations of plant growth regulators in culture media on shoot proliferation from shoot tip explants of MM106 apple rootstock were tested. The basic salts and vitamins of Murashige and Skoog (1962) (MS) were used at full strength for the *in vitro* shoot proliferation. The culture media were supplemented with sucrose at the rate of $30g1^{-1}$. Bacto difco agar was added at the rate of $7g1^{-1}$. Plant growth regulators included benzyladenine (BA) at 0.0, 1.0, 2.0 and 3.0 $mg1^{-1}$ and α - naphthalene acetic acid (NAA) at 0.0, 0.1, 0.2 and 0.3 $mg1^{-1}$ were supplemented solely or in various combinations and concentrations in MS media.

The aseptic explants were cultured vertically in glass test tubes (25×150 mm) containing 10 ml (each) of shoot proliferation media. One shoot tip (explant) cultured in test tube. The test tubes were covered with cotton and capped with aluminum foil. The pH of shoot proliferation media was adjusted to 5.7 using NaOH and HCl before adding agar. These media sterilized in an autoclave at 121°C for 20 min., then left to cool and harden for 24 hrs before being used.

1.4. Culture Conditions

All the shoot tip cultures were maintained in growth culture room under controlled conditions, at 25±2°C, in a 16 hrs light period from fluorescent lamps (2 lamps per shelf), followed by 8 hrs dark period.
The shoot proliferation percentage calculated as follows:

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

The shoot proliferation percentage, average number of proliferated shoots per shoot tip explant and average length (cm) of proliferated shoot were recorded after 4 weeks of explant culture. The shoot proliferation rate = number of proliferated shoots per shoot tip (explant) in shoot tip cultures after 4 weeks of culture. The resultant proliferated shoots (axillary shoots) were used as a mother stock explants for the shoot multiplication experiments.

1.5. Statistical Analysis

In all experiments of shoot proliferation, each treatment consisted of three replicates with ten explants (shoot tips) each in a completely randomized design and the statistical procedures were applied according to Steel and Torrie (1980) The combinations between BA and NAA concentrations in MS culture media were represented by 16 combinations as indicated in Table (1) and took the combination code from C_1 to C_{16}.

2. Shoot Multiplication

These experiments were performed to study the influence of cytokinins benzyladenine (BA) and kinetin (Kin) combinations in culture

media on the shoot multiplication in the shoot cultures of MM106 apple rootstock.

2.1. Plant Material

The proliferated shoots that were produced from the shoot tip cultures (the former experiments) were excised under aseptic conditions and transfered (individually) to culture tubes containing the shoot multiplication media.

2.2. Culture Media

The basic salts and vitamins of Murashige and Skoog (1962) (MS) were used at full strength for the *in vitro* shoot multiplication. The culture media were supplement with sucrose at the rate of $30g1^{-1}$. Bacto difco agar was added at the rate of $7g1^{-1}$. Plant growth regulators included benzyladenine (BA) at 0.0, 0.5, 1.0 and 2.0 $mg1^{-1}$ and kinetin (Kin) at the same concentration 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 5.7 before adding agar. The culture tubes (25×150 mm) filled with 10ml (each) of shoot multiplication media, covered with cotton and capped with aluminum foil. The culture media autoclaved at 121°C for 20 min, then left to cool and harden for 24 hr before being used. Routine subculture of axillary shoots was carried out every 4 weeks up to four subcultures. One original shoot cultured in test tube. At the end of each subculture (after 4 weeks), the new proliferated shoots were used as a mother cultures for the subsequent subculture, individually separated and transfered to a fresh multiplication medium.

2.3 . Culture Conditions

All the shoot cultures were grown at 25±2°C, in a 16/8 hrs light/dark cycle with fluorescent light.

The shoot multiplication percentage calculated as follows:

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

The percentage of cultures with multiple shoots, average number of new proliferated shoots (axillary shoots) that was produced from each original shoot and the average length (cm) of new proliferated shoots was recorded at the end of each subculture (after 4 weeks of original shoot culture). The resultant proliferated shoots from each subculture were used as a mother stock explants for the next subculture.

The shoot multiplication rate = number of new proliferated shoots per original shoot in shoot cultures at the end of each subculture (4 weeks). The new proliferated shoots were used as a mother cultures for the subsequent rooting experiments.

2.4. Statistical Analysis

In all experiments of shoot multiplication, each treatment consisted from three replicates with ten original shoots for each in a completely

randomized design and the statistical procedures were applied according to Steel and Torrie (1980). The combinations between BA and Kin concentrations in MS culture media were represented by 16 combinations as indicated in Tables (2 to 4) and took the combination code from C_1 to C_{16}

RESULTS AND DISCUSSION

1. Shoot Proliferation

Data concerning the effect of BA and NAA combinations on shoot proliferation percentage from shoot tip explants, average number of proliferated shoots per explant and average length (cm) of proliferated shoot of MM 106 apple rootstock, are listed in Table (1).

1.1. Effect of BA and NAA combinations on the shoot proliferation percentage

The results in Table (1) indicated that, shoot proliferation percentage from shoot tip explant was significantly the highest (96.67%) on 2.0 $mg1^{-1}$ BA + 0.2 $mg1^{-1}$ NAA combination (C_{11}). On the contrary, the lowest percentage (10.00%) was resulted in 0.0 $mg1^{-1}$ BA + 0.1 $mg1^{-1}$ NAA combination (C_5). The data showed no shoot proliferation occurred (0.00%) on the MS culture medium without the addition of growth regulators (C_1).

1.2. Effect of BA and NAA combinations on average number of proliferated shoots per explant

In respect to the effect of BA and NAA combinations on average number of proliferated shoots per shoot tip explant, the results in Table (1) indicated that, average number of proliferated shoots per shoot tip explant was significantly the highest (7.79) on 2.0 $mg1^{-1}$ BA + 0.2 $mg1^{-1}$ NAA combination (C_{11}). In contrast, the lowest average number (1.00) was resulted in 1.0 $mg1^{-1}$ BA + 0.0 $mg1^{-1}$ NAA combination (C_2), 2.0 $mg1^{-1}$ BA + 0.0 $mg1^{-1}$ NAA combination (C_3), 0.0 $mg1^{-1}$ BA + 0.1 $mg1^{-1}$ NAA combination (C_5), 0.0 $mg1^{-1}$ BA + 0.2 $mg1^{-1}$ NAA combination (C_9) and 0.0 $mg1^{-1}$ BA + 0.3 $mg1^{-1}$ NAA combination (C_{13}) On the other side, the data showed no shoot proliferation occurred (0.00 %) on the MS culture medium without the addition of growth regulators (C_1).

1.3. Effect of BA and NAA combinations on average length of proliferated shoot

Results in Table (1) indicated that, average length of proliferated shoot was significantly the highest (2.36 cm) on 2.0 $mg1^{-1}$ BA + 0.2 $mg1^{-1}$ NAA combination (C_{11}) On the contrary, the lowest average length (0.55 cm) was recorded with 0.0 $mg1^{-1}$ BA + 0.1 $mg1^{-1}$ NAA combination (C_5). The same results showed no shoot proliferation occurred (0.00%) on the MS culture medium without the addition of growth regulators (C_1).

Table (1): Effect of BA and NAA combinations on shoot proliferation percentage, average number of proliferated shoots per explant and average length (cm) of proliferated shoot of MM106 apple rootstock cultures.

Combination code	Growth regulators in $mg1^{-1}$		Shoot proliferation (%)	Av. no. of proliferated shoots/explant	Av. length (cm) of proliferated shoot
	BA	NAA			
C_1	0.0	0.0	0.00 P	0.00 J	0.00 M
C_2	1.0	0.0	20.00 L	1.00 I	1.00 J
C_3	2.0	0.0	23.33 K	1.00 I	1.13 I
C_4	3.0	0.0	26.67 J	1.13 HI	1.36 H
C_5	0.0	0.1	10.00 O	1.00 I	0.55 L
C_6	1.0	0.1	46.67 G	1.71 G	1.67 F
C_7	2.0	0.1	40.00 H	1.50 G	1.55 G
C_8	3.0	0.1	36.67 I	1.27 H	1.40 H
C_9	0.0	0.2	13.33 N	1.00 I	0.64 L
C_{10}	1.0	0.2	86.67 B	6.19 B	2.12 B
C_{11}	2.0	0.2	96.67 A	7.79 A	2.36 A
C_{12}	3.0	0.2	73.33 C	5.00 C	2.00 C
C_{13}	0.0	0.3	16.67 M	1.00 I	0.84 K
C_{14}	1.0	0.3	50.00 F	2.00 F	1.75 EF
C_{15}	2.0	0.3	53.33 E	3.19 E	1.82 DE
C_{16}	3.0	0.3	60.00 D	4.39 D	1.90 D
L.S.D. 0.05			1.565	0.229	0.094

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

From the overall results it is evident that *in vitro* shoot proliferation of MM 106 apple rootstock could be achieved successfully through the development of axillary shoots from shoot tip explants as a starting plant material. These findings are in agreement with those reported by Bondok *et al.* (1987); Yui *et al.* (1993); Turovskaya (1994); ShuiTao *et al.* (1996); XiaoXin *et al.* (1997); Khan *et al.* (1998) and JunBao *et al.* (1999).

The results indicated that the combination of cytokinin (BA) and auxin (NAA) appeared to be essential for shoot proliferation in MM 106 apple rootstock cultures. It is worthy to mention that shoot proliferation of MM 106 apple rootstock was accomplished on Murashige and Skoog (1962) medium supplemented with BA combined with NAA. The highest values of the shoot proliferation percentage from shoot tip explant (96.67%), average number of proliferated shoots per shoot tip explant (7.79) and average length of proliferated shoot (2.36 cm) obtained on MS medium + 2.0 $mg1^{-1}$ BA + 0.2 $mg1^{-1}$ NAA. These results are in accordance with those found by Machnik and Iisek (1995). They reported that MS medium containing 0.75 $mg1^{-1}$ BA + 0.05 $mg1^{-1}$ NAA was used as the shoot proliferation medium in apple rootstocks (P. 16 , P. 22, P. 14, P. 2 and P. 59).

Such findings also partially agreed with those reported by Bondok *et al.*, (1987). They obtained the proliferated shoots from shoot tips of MM 106 apple rootstock, "Anna" and "Baladi" apple cultivars when were cultured on the Murashige and Skoog (1962) medium containing 6.0 $mg1^{-1}$ BA. On the same medium, the shoot proliferation rate was 6.25 shoot / explant (overall the average ranged from 2.7 to 14.0). Moreover, Yui *et al.*, (1993) found that *in vitro* shoot proliferation in meristem cultures of M.7 apple rootstock could be successfully using BA alone at 0.5 – 2.0 $mg1^{-1}$. Also, Turovskaya (1994) reported that shoot proliferation of apple rootstocks in MS medium depended on BAP concentration, with 2-3 $mg1^{-1}$ being optimum for stocks On the same medium, the shoot proliferation rate was 2.7 – 5.0 shoot per explant. Furthermore, ShuiTao *et al.*, (1996) mentioned that, the best culture medium for shoot proliferation of "Fuji" apple was MS medium supplemented with 1.0 $mg1^{-1}$ benzyladenine and 0.1 $mg1^{-1}$ IBA.

In this concept, Aklan *et al.*, (1997) reported that half – strength MS medium containing 2.2 µM BA, 0.5 µM IBA and 0.3 µM GA_3 was used as the shoot proliferation medium in meristems of MM.106 apple rootstock. The rate of shoot proliferation / explant was 3.4. In the meantime, XiaoXin *et al.*, (1997) mentioned that, the shoot proliferation in shoot tips of 3 apple cultivars was the best on culture medium containing 0.5 $mg1^{-1}$ BA + 0.2 $mg1^{-1}$ IBA. HaoRu *et al.* (1998) found that Murashige and Skoog (1962) medium supplemented with BA at 2.0 $mg1^{-1}$ and IBA at 0.2 $mg1^{-1}$ was used as the shoot proliferation medium in shoot tips of Golden Delicious apple. Besides, Khan *et al.* (1998) reported that, sprouting frequency of shoot proliferation in bud explants of apple cv. MM. 106 was the highest (73.75%) on MS medium supplemented with BA at 1.5 $mg1^{-1}$.

Recently, JunBao *et al.* (1999) noticed that, MS medium supplemented with BA (0-15 $mg1^{-1}$), NAA (0 -5 $mg1^{-1}$), kinetin (0 – 0.5 $mg1^{-1}$) and / or 2,4-D (0 – 5 $mg1^{-1}$) was used as the shoot proliferation medium in shoot tips of apple cv. Fuji. In addition, Modgil *et al.* (1999) reported that the buds of apple cv. Tydeman's Early Worcester were grown to proliferated shoots on MS medium + 4.4 µM BA + 2.8 µM GA_3 + 0.5 µM IBA.

2. Shoot Multiplication

Regarding the effect of BA and Kin. combinations on shoot multiplication from original shoot (derived from shoot tip cultures) through four subsequent subcultures (after 4, 8, 12 and 16 weeks of original shoot culture), data are listed in Tables (2 to 4).

2.1. Effect of BA and Kin. combinations on the percentage of cultures with multiple shoots

The results in Table (2) indicated that, in the first subculture (after 4 weeks of original shoot culture), the percentage of cultures with multiple shoots was significantly the highest (90.00%) on 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_7). On the contrary, the lowest percentage (3.33%) was achieved in 0.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_5). The data also showed no multiplicated shoots occurred (0.00%) on the MS medium without

the addition of growth regulators (C_1). Similar trend was noticed in the 2nd, 3rd and 4th subcultures.

The results cleared that, in the second subculture (after 8weeks of original shoot culture), the percentage of cultures with multiple shoots was significantly the highest (96.67%) on 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_7). In contrast, the lowest percentage (6.67%) was resulted in 0.0 $mg1^{-1}$ BA +0.5 $mg1^{-1}$ Kin. combination (C_5).

The data, also, indicated that, in the third subculture (after 12 weeks of original shoot culture), the percentage of cultures with multiple shoots was significantly the highest (100.00%) on 1.0 $mg1^{-1}$ BA+ 0.5 $mg1^{-1}$ Kin. combination (C_7). On the contrary, the lowest percentage (10.00%) was obvious in 0.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_5).

As for, the fourth subculture (after 16 weeks of original shoot culture), the results revealed that, the percentage of cultures with multiple shoots was significantly the highest (93.33%) on 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_7). In contrast, the lowest percentage (6.67%) was achieved with 0.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_5).

Table (2): Effect of BA and Kin. combinations on the percentage of cultures with multiple shoots (proliferated from shoot cultures) of MM106 apple rootstock.

Combination code	Growth regulators in $mg1^{-1}$		Cultures with multiple proliferated shoots[y] (%)			
	BA	Kin	1st Subculture	2nd Subculture	3rd Subculture	4th Subculture
C_1	0.0	0.0	0.00 [x]P*	0.00 P	0.00 P	0.00 P
C_2	0.5	0.0	16.67 L	23.33 L	26.67 L	20.00 L
C_3	1.0	0.0	23.33 K	30.00 K	33.33 K	26.67 K
C_4	2.0	0.0	30.00 J	36.67 J	40.00 J	33.33 J
C_5	0.0	0.5	3.33 O	6.67 O	10.00 O	6.67 O
C_6	0.5	0.5	70.00 D	76.67 D	80.00 D	73.33 D
C_7	1.0	0.5	90.00 A	96.67 A	100.00 A	93.33 A
C_8	2.0	0.5	63.33 E	70.00 E	73.33 E	66.67 E
C_9	0.0	1.0	6.67 N	13.33 N	16.67 N	10.00 N
C_{10}	0.5	1.0	36.67 I	43.33 I	46.67 I	40.00 I
C_{11}	1.0	1.0	83.33 B	90.00 B	93.33 B	86.67 B
C_{12}	2.0	1.0	76.67 C	83.33 C	86.67 C	80.00 C
C_{13}	0.0	2.0	10.00 M	16.67 M	20 00 M	13.33 M
C_{14}	0.5	2.0	43.33 H	50.00 H	53.33 H	46.67 H
C_{15}	1.0	2.0	50.00 G	56.67 G	60.00 G	53.33 G
C_{16}	2.0	2.0	56.67 F	63.33 F	66.67 F	60.00 F
L.S.D. 0.05			0 832	0.832	0.720	0.720

[y]Values refer to the percentage of original shoots that produced multiple shoots.
[x]Zero values indicate absence of multiple shoots.
*Values followed by the same letters (in the same column) significantly are not differed at the 0.05 level of probability.

2.2. Effect of BA and Kin. combinations on average number of new proliferated shoots

The results in Table (3) indicated that, in the first subculture, average number of new proliferated shoots per original shoot was significantly the highest (7.52) on 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_7). On the contrary, the lowest average number (2.00) was achieved in 0.5 $mg1^{-1}$ BA + 0.0 $mg1^{-1}$ Kin. combination (C_2), 0.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin combination (C_5), 0.0 $mg1^{-1}$ BA + 1.0 $mg1^{-1}$ Kin. combination (C_9) and 0.0 $mg1^{-1}$ BA +2 0 $mg1^{-1}$ Kin. combination (C_{13}). The results also showed no multiplicated shoots occurred (0.00) on the MS medium without the addition of growth regulators (C_1). Similar trend was noticed in the 2nd, 3rd and 4th subcultures.

The results cleared that, in the second subculture, average number of new proliferated shoots per original shoot was significantly the highest (9. 79) on 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_7). In contrast, the lowest average number (2.50) was resulted in 0.0 $mg1^{-1}$ BA+ 0.5 $mg1^{-1}$ Kin combination (C_5).

The data, also, indicated that, in the third subculture, average number of new proliferated shoots per original shoot was significantly the highest (10.00) on 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_7). On the contrary, the lowest average number (3.00) was obvious in 0.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_5).

As for, the fourth subculture, the results revealed that, average number of new proliferated shoots per original shoot was significantly the highest (8.21) on 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_7). In contrast, the lowest average number (2.00) was achieved with 0.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_5).

Table (3): Effect of BA and Kin. combinations on average number of new proliferated shoots per original shoot of MM106 apple rootstock cultures.

Combination code	Growth regulators in $mg1^{-1}$		Av. no. of new proliferated shoots/ original shoot			
	BA	Kin	1st Subculture	2nd Subculture	3rd Subculture	4th Subculture
C_1	0.0	0.0	0.00 L	0.00 P	0.00 P	0.00 P
C_2	0.5	0.0	2.00 K	3.86 L	4.75 L	2.67 L
C_3	1.0	0.0	2.43 J	4.00 K	5.00 K	3.25 K
C_4	2.0	0.0	2.56 I	4.09 J	5.17 J	3.60 J
C_5	0.0	0.5	2.00 K	2.50 O	3.00 O	2.00 O
C_6	0.5	0.5	5.19 D	7.09 D	8.42 D	6.59 D
C_7	1.0	0.5	7.52 A	9.79 A	10.00 A	8.21 A
C_8	2.0	0.5	4.58 E	6.29 E	7.50 E	5.40 E
C_9	0.0	1.0	2.00 K	2.75 N	3.40 N	2.33 M
C_{10}	0.5	1.0	2. 91 H	4.39 I	5.43 I	3.83 I
C_{11}	1.0	1.0	6.12 B	8.00 B	9.50 B	7.81 B
C_{12}	2.0	1.0	5.91 C	7.72 C	8.81 C	6.71 C
C_{13}	0.0	2.0	2.00 K	3.60 M	4.50 M	2.25 N
C_{14}	0.5	2.0	3.00 H	5.00 H	6.19 H	4.29 H
C_{15}	1.0	2.0	3.60 G	5.41 G	6.78 G	4.63 G
C_{16}	2.0	2.0	4.00 F	6.00 F	7.20 F	5.00 F
L.S.D. 0.05			0.095	0.084	0.046	0.045

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

2.3. Effect of BA and Kin. combinations on average length of new proliferated shoot

The results in Table (4) indicated that, in the first subculture, average length of new proliferated shoot was significantly the highest (2.65 cm) on 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_7). On the contrary, the lowest average length (1.20 cm) was noticed in 0.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_5). The results also showed no multiplicated shoots occurred (0.00) on the MS medium without the addition of growth regulators (C_1). The same trend was obvious in the 2nd, 3rd and 4th subcultures.

The results indicated that, in the second subculture, average length of new proliferated shoot was significantly the highest (2.83 cm) on 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_7). In contrast, the lowest average length (1.27 cm) was resulted in 0.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_5).

The same data indicated that, in the third subculture, average length of new proliferated shoot was significantly the highest (3.00 cm) on 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_7). On the contrary, the lowest average length (1.36 cm) was resulted in 0.0 $mg1^{-1}$ BA +0.5 $mg1^{-1}$ Kin. combination (C_5).

As for, the fourth subculture, the results revealed that, average length of new proliferated shoot was significantly the highest (2.70 cm) on 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin. combination (C_7). In contrast, the lowest average length (1.20 cm) was resulted in 0.5 $mg1^{-1}$ BA +0.0 $mg1^{-1}$ Kin. combination (C_2).

Table (4): Effect of BA and Kin. combinations on average length (cm) of new proliferated shoot of MM106 apple rootstock cultures.

Combination Code	Growth regulators in $mg1^{-1}$		Av. length (cm) of new proliferated shoot			
	BA	Kin	1st Subculture	2nd Subculture	3rd Subculture	4th Subculture
C_1	0.0	0.0	0.00 K	0.00 M	0.00 L	0.00 L
C_2	0.5	0.0	1.30 I	1.33 K	1.42 K	1.20 K
C_3	1.0	0.0	1.40 H	1.44 J	1.56 I J	1.32 J
C_4	2.0	0.0	1.41 H	1.50 I	1.62 I	1.47 I
C_5	0.0	0.5	1.20 J	1.27 L	1 36 K	1.23 K
C_6	0.5	0.5	1.82 D	2.04 D	2.25 D	2.12 D
C_7	1.0	0.5	2.65 A	2.83 A	3.00 A	2.70 A
C_8	2.0	0.5	1.72 E	1.90 E	2.18 E	2.10 D
C_9	0.0	1.0	1.33 I	1.44 J	1.50 J	1.33 J
C_{10}	0.5	1.0	1.54 G	1.62 H	1.77 H	1.54 H
C_{11}	1.0	1.0	2.40 B	2.63 B	2.82 B	2.50 B
C_{12}	2.0	1.0	2.00 C	2.25 C	2.47 C	2.32 C
C_{13}	0.0	2.0	1.30 I	1.45 J	1.52 J	1.34 J
C_{14}	0.5	2.0	1.55 G	1.72 G	1.86 G	1.65 G
C_{15}	1.0	2.0	1.64 F	1.73 G	1.90 G	1 80 F
C_{16}	2.0	2.0	1.60 F	1.85 F	2.10 F	1.92 E
L.S.D. 0.05			0.048	0.031	0.064	0.030

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

The obtained results clearly proved that shoot multiplication of MM 106 apple rootstock could be successfully achieved through four subsequent subcultures from shoot explant cultures. These findings are in agreement with those reported by El-Wakeel (1991), Shawky *et al* (1993) and Modgil *et al* (1999).

The same results showed that, the combination of BA and Kin appeared to be essential for shoot multiplication in MM 106 apple rootstock cultures. It is evident that shoot multiplication in MM 106 apple rootstock was accomplished on Murashige and Skoog (1962) medium supplemented with BA combined with Kin. The highest values of cultures percentage with multiple shoots from original shoot, average number of new proliferated shoots per original shoot and average length of new proliferated shoot obtained on MS medium + 1.0 $mg1^{-1}$ BA + 0.5 $mg1^{-1}$ Kin . These findings are in accordance with those reported by Modgil *et al.*, (1999). They found that the shoots of apple cv. Tydeman's Early Worcester were multiplied on MS medium with 2.2 µM BA and 7.5 µM Kinetin.

The present results also partially agreed with those reported by El-Wakeel (1991). Who found that BAP (1 $mg1^{-1}$) gave higher significant shoot multiplication rate for M26 apple rootstock shoots through four successive subcultures. Furthermore, Shawky *et al.* (1993) reported that the highest shoot multiplication rate for M26 apple rootstock shoots could be achieved by using MS basal medium supplemented with BAP at 1.0 $mg1^{-1}$ + 162 $mg1^{-1}$ phloroglucinol (PG) for the first two subcultures.

On the other hand, the results of the third subculture which gave the highest values of the percentage of cultures with multiple shoots from original shoot (100.00%), average number of new proliferated shoots per original shoot (10.00) and average length of new proliferated shoot (3.00 cm) compared to the other subcultures. These findings disagreed with those obtained by El-Wakeel (1991) and Shawky *et al.* (1993) who reported that the first two subcultures gave the highest values of shoot multiplication rate in M 26 apple rootstock compared to those of the subsequent subcultures (third, fourth and fifth subcultures).

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الإكثار المعملى الدقيق لأصل التفاح مولنج مورتن ١٠٦

١- إنتاج الأفرخ وإكثارها

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أجرى هذا البحث خلال ثلاث سنوات متتالية (٢٠٠٠- ٢٠٠٢) بغرض الإكثار المعملى الدقيق لأصل التفاح مولنج مورتن ١٠٦ باستخدام تقنية زراعة الأنسجة وذلك بدراسة تأثير بيئة الزراعة موراشيج وسكوج (١٩٦٢) مضافا إليها تركيزات مختلفة من منظمات النمو النباتية على إنتاج الأفرخ من منفصل القمة النامية وإكثار هذه الأفرخ الناتجة فى مزارع الأنسجة:

ويمكن تلخيص النتائج الرئيسية لهذه الدراسة فى النقاط التالية:-

١- قيم النسبة المئوية لإنتاج الأفرخ من منفصل القمة النامية ومتوسط عدد الأفرخ الناتجة من كل منفصل للقمة النامية ومتوسط طول الفرخ الناتج كانت مرتفعة بصورة جوهرية (٩٦,٦٧% ، ٧,٧٩ ، ٢,٣٦سم على التوالى) وذلك على بيئة موراشيج وسكوج (١٩٦٢) + ٢,٠ ملجرام فى اللتر بنزايل أدنين + ٠,٢ ملجرام فى اللتر نفثالين حمض الخليك.

٢- أدى استخدام بيئة موراشيج وسكوج (١٩٦٢) مضاف إليها ١,٠ ملجرام فى اللتر بنزايل أدنين + ٠,٥ ملجرام فى اللتر كينتين إلى الحصول على أعلى قيم للنسبة المئوية للمزارع المحتوية على أفرخ متعددة جديدة ناتجة من الفرخ الأصلى ومتوسط عدد الأفرخ الجديدة الناتجة من كل فرخ أصلى ومتوسط طول الفرخ الناتج الجديد وذلك خلال أربعة زراعات (نقلات) متتالية.

٣- ففى النقلة الأولى والثانية والثالثة والرابعة، كانت قيمة النسبة المئوية للمزارع المحتوية على أفرخ متعددة ناتجة من الفرخ الأصلى مرتفعة بصورة جوهرية (٩٠,٠٠% ، ٩٦,٦٧% ، ١٠٠,٠٠% ، ٩٣,٣٣% على التوالى). وكانت قيمة متوسط عدد الأفرخ الجديدة الناتجة من كل فرخ أصلى مرتفعة بصورة جوهرية (٧,٥٢ ، ٩,٧٩ ، ١٠,٠٠ ، ٨,٢١ على التوالى). وكانت قيمة متوسط طول الفرخ الجديد الناتج مرتفعة بصورة جوهرية (٢,٦٥ سم ، ٢,٨٣ سم ، ٣,٠٠ سم ، ٢,٧٠ سم على التوالى).