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MICROPROPAGATION OF MALLING MERTON106 APPLE ROOTSTOCK : II- ROOTING AND ACCLIMATIZATION

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

ABSTRACT

The present research was carried out during three successive years (2000-2002), in order to study the effect of indole butyric acid (IBA) to phloroglucinol (PG) ratio in culture medium [Murashige and Skoog (1962) at half strength] on rooting of shoots and subsequently, acclimatization of the obtained plantlets of Malling Merton 106 (MM 106) apple rootstock.

The main results can be summarized in the following points:

1- Using the Murashige and Skoog (1962) medium at half strength (1/2 MS) containing 1.0 $mg1^{-1}$ IBA and 162.0 $mg1^{-1}$ PG, the rooting percentage of shoots was significantly the highest (100%). The average number of roots per shoot (7.6) and the average root length per shoot (4.67 cm) were significantly the highest.

2- Seventy-five percent of the obtained plants of MM 106 apple rootstock were successfully transplanted to soil. These plants were uniformity, vigorously growing and healthy under the greenhouse conditions.

INTRODUCTION

The apples (*Malus domestica* Borkh.) are one of the most important economic fruit crops in Egypt. The total apple cultivated area reached 65141 feddans producing about 473588 tons of fruits according to the statistics of the Ministry of Agriculture and Land Reclamation, Cairo, 2001.

The increase of the new established apple orchards mostly is concentrated in Nubaria region (new cultivated area). "Anna" is the most widely apple variety cultivated in Egypt, especially in the new reclaimed land. The suitable apple 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 such 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).

Micropropagation *in vitro* involves the culture of explants and production of shoots through adventitious or axillary shoot proliferation. These shoots are then induced to form adventitious roots, usually by culture on a medium containing an auxin (Nemeth, 1986). Rooted shoots are transferred to a greenhouse and then to a field.

Apple cultivars are difficult to root, even under *in vitro* conditions (Sriskandarajah and Mullins, 1981). Any improvement made concerning root induction *in vitro* will be beneficial for plant establishment *ex vitro*.

Previous reports on apple tissue culture concentrated on factors such as temperature, light and phloroglucinol and their influence on rooting (Sriskandarajah *et al.*, 1982 and Travers *et al.*, 1985).

The efficient and reliable production of vigorously growing plants in soil from *in vitro* plant material is an important step in the evaluation of apple cultivars. Research on the factors involved in the development of effective rooting techniques has yielded variable success (Zimmerman, 1984 and; Zimmerman and Fordham, 1985).

This work aimed to study the effect of IBA and PG combinations on rooting of shoots and subsequently, acclimatization of the obtained plantlets of MM 106 apple rootstock.

MATERIALS AND METHODS

The present investigation was conducted during three successive years (2000-2002), in order to study the possibility of using tissue culture technique for rapid and economical micropropagation of Malling Merton 106 (MM 106) (*Malus pumila* Mill.) apple rootstock.

The objective of this study was to determine the optimal culture conditions for *in vitro* rooting of multiplicated shoots of MM 106 rootstock. This work examined the effect of various combinations and concentrations of indole -3- butyric acid (auxin) and phloroglucinol (phenolic compound) in culture media on rooting of MM 106 apple shoot cultures.

1. Rooting of Proliferated Shoots

1.1. Plant Material

These experiments were carried out on proliferated shoots of MM 106 apple rootstock derived from *in vitro* shoot multiplication.

1.2. Culture Media

The basic salts and vitamins of Murashige and Skoog (1962) (MS) were used at half salts strength (1/2 MS) for rooting media. At the end of each subculture, uniformity, vigorously growing and healthy proliferated shoots (≥ 1.20 cm in length) were excised and transferred (individually) under aseptical conditions and inoculated vertically into culture tubes (25 × 150 mm) containing 10 ml (each) of 1/2 MS basal medium amended with 2% sucrose and 0.6% agar. Indole –3- butyric acid (IBA) at 0.0, 1.0, 2.0 and 3.0 $mg1^{-1}$ and phloroglucinol (PG) at 0.0, 40.5, 81.0 and 162.0 $mg1^{-1}$ were supplemented solely or in various combinations and concentrations in 1/2 MS

rooting media. The pH of the rooting media was adjusted to 5.7 before adding agar. The culture tubes closed with cotton, capped with aluminum foil, and sterilized in an autoclave at 121°C for 20 min, then left to cool and harden for 24 hrs before being used. One proliferated shoot cultured in culture tube. Rooting percentage, average number of roots per shoot and average root length per shoot were recorded after 4 weeks of shoot culture. The rooting percentage calculated as follows:

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

1.3. Culture Conditions

The shoot cultures were incubated on racks in growth culture room at a temperature of 25 ± 2 °C, with 16 hrs photoperiod provided by white fluorescent tubes, followed by 8 hrs dark period for 4 weeks.

1.4. Statistical Analysis

In rooting experiments, each treatment consisted of three replicates with ten shoots each in a completely randomized design and the statistical procedures were applied according to Steel and Torrie (1980).

The combinations between IBA and PG concentrations in 1/2 MS culture media were represented by 16 combinations as indicated in Tables (1 to 3) and took the combination code from C_1 to C_{16}.

2. Transplanting of Plants to Soil

The obtained plantlets (healthy and vigorously) were rinsed in water to remove any medium, misted with water to prevent wilting, and then transferred to plastic pots containing a sterilized mixture of peat moss: perlite (1: 2 v/v). The pots were watered, covered with plastic bags, and placed in growth chamber at 70 to 80% relative humidity, at 23 ± 2 °C, under white fluoresent tube lights (16 hrs photoperiod).

To acclimate the plants, after 4 days or when growth of a new leaf was observed, the corner of the plastic bag was cut with scissor, and 4 days later the bag was removed. The pots were watered regularly and fertilized weekly with the addition of appropriate volume of nutrient 1/2 MS medium without sucrose. The plants were then transferred to a greenhouse, for 3 months. Observations on survival and growth were recorded.

RESULTS AND DISCUSSION

1. Rooting of Proliferated Shoots

Data concerning the effect of IBA and PG combinations on the rooting percentage of proliferated shoots (derived from shoot multiplication experiments), average number of roots per shoot and average root length per shoot of MM106 apple rootstock, are listed in Tables (1 to 3).

1.1. Effect of IBA and PG combinations on the rooting percentage

The results in Table (1) indicated that, the rooting percentage of proliferated shoots (obtained from shoot multiplication experiments) was significantly the highest (100.00%) on 1.0 $mg1^{-1}$ IBA + 162.0 $mg1^{-1}$ PG combination (C_{14}). On the contrary, the lowest percentage (6.67%) was resulted in 0.0 $mg1^{-1}$ IBA + 40.5 $mg1^{-1}$ PG combination (C_5).

On the other hand, the data showed no rooted shoots occurred (0.00%) on 1/2 MS culture medium without the addition of IBA and PG (C_1).

Table (1): Effect of IBA and PG combinations on the rooting percentage of proliferated shoots (derived from original shoot cultures) of MM106 apple rootstock.

Combination code	($mg1^{-1}$)		Proliferated shoots formed roots[y] (Rooting %)
	IBA	PG	
C_1	0.0	00.0	0.00[x] P[*]
C_2	1.0	00.0	16.67 L
C_3	2.0	00.0	23.33 K
C_4	3.0	00.0	30.00 J
C_5	0.0	40.5	6.67 O
C_6	1.0	40.5	36.67 I
C_7	2.0	40.5	43.33 H
C_8	3.0	40.5	46.67 G
C_9	0.0	81.0	10.00 N
C_{10}	1.0	81.0	53.33 F
C_{11}	2.0	81.0	56.67 E
C_{12}	3.0	81.0	60.00 D
C_{13}	0.0	162.0	13.33 M
C_{14}	1.0	162.0	100.00 A
C_{15}	2.0	162.0	86.67 B
C_{16}	3.0	162.0	70.00 C
$L.S.D_{0.05}$			1.611

[y] Values refer to the percentage of proliferated shoots that produced roots.
[x] Zero values indicate absence of roots.
[*] Values followed by the same letters significantly are not differed at the 0.05 level of probability.

1.2. Effect of IBA and PG combinations on average number of roots per shoot

In respect to the effect of IBA and PG combinations on average number of roots per shoot, the results in Table (2) indicated that, average number of roots per shoot was significantly the highest (7.60) on 1.0 $mg1^{-1}$ IBA + 162.0 $mg1^{-1}$PG combination (C_{14}), whereas, the lowest average number (1.00) was resulted in 1.0 $mg1^{-1}$ IBA + 0.0 $mg1^{-1}$ PG combination (C_2), 0.0 $mg1^{-1}$ IBA + 40.5 $mg1^{-1}$ PG combination (C_5), 0.0 $mg1^{-1}$ IBA + 81.0 $mg1^{-1}$ PG combination (C_9) and 0.0 $mg1^{-1}$ IBA + 162.0 $mg1^{-1}$PG combination (C_{13}).

On the other side, the results showed no rooted shoots occurred on 1/2 MS culture medium without the addition of IBA and PG (C_1).

Table (2): Effect of IBA and PG combinations on average number of roots per proliferated shoot (derived from original shoot cultures) of MM106 apple rootstock.

Combination code	($mg1^{-1}$)		Average number of roots/ shoot
	IBA	PG	
C_1	0.0	00.0	0.00 M
C_2	1.0	00.0	1.00 L
C_3	2.0	00.0	1.43 K
C_4	3.0	00.0	1.56 J
C_5	0.0	40.5	1.00 L
C_6	1.0	40.5	1.82 I
C_7	2.0	40.5	2.00 H
C_8	3.0	40.5	2.57 G
C_9	0.0	81.0	1.00 L
C_{10}	1.0	81.0	3.00 F
C_{11}	2.0	81.0	4.53 E
C_{12}	3.0	81.0	5.00 D
C_{13}	0.0	162.0	1.00 L
C_{14}	1.0	162.0	7.60 A
C_{15}	2.0	162.0	6.39 B
C_{16}	3.0	162.0	6.19 C
$L.S.D_{0.05}$			0.118

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

1.3. Effect of IBA and PG combinations on average root length

Results in Table (3) indicated that, average length of root per shoot was significantly the highest (4.67 cm) on 1.0 $mg1^{-1}$ IBA + 162.0 $mg1^{-1}$ PG combination (C_{14}). On the contrary, the lowest average length (0.45 cm) was recorded with 0.0 $mg1^{-1}$ IBA + 40.5 $mg1^{-1}$ PG combination (C_5), whereas, the data showed no rooted shoots occurred on 1/2 MS culture medium without the addition of IBA and PG (C_1).

From the overall results it is evident that *in vitro* rooting of proliferated shoots of MM106 apple rootstock could be achieved successfully through the formation of adventitious roots from shoot cultures.

These findings are in agreement with those reported by Webster and Jones (1991), Shawky *et al.* (1993), Correa *et al.* (1994), Aklan *et al.* (1997), Ferri *et al.* (1998) and Isutsa *et al.* (1998).

The same results indicated that the combination of IBA and PG appeared to be essential for rooting of proliferated shoots in MM106 apple rootstock cultures. The highest values of the rooting percentage (100%), average number of roots per shoot (7.6) and average root length (4.67 cm) obtained on 1/2 MS medium + 1.0 $mg1^{-1}$ IBA + 162.0 $mg1^{-1}$ PG. These findings are in accordance with those reported by Welander (1983), who found that, rooting of M26 apple shoots was the highest (66 – 70%) with

adding 15.0 µM IBA + 10.0 µM PG to MS medium. In the meantime, Aklan *et al.*, (1997) reported that the best rooting (90%) for proliferated shoots of MM106 apple rootstock was achieved with 5.0 µM IBA plus 162.0 $mg1^{-1}$ phloroglucinol. In addition, Zanol *et al.* (1998) reported that phloroglucinol in the presence of IBA accelerated rooting and increased the rooting percentage. They also mentioned that the maximum rooting of proliferated shoots in the apple rootstock Marubakaido, occurred in rooting medium contained 1.0 µM IBA plus 162.0 $mg1^{-1}$ phloroglucinol.

Table (3): Effect of IBA and PG combinations on average length (cm) of root per proliferated shoot (derived from original shoot cultures) of MM106 apple rootstock.

Combination code	($mg1^{-1}$)		Average root length (cm)
	IBA	PG	
C_1	0.0	00.0	0.00 O*
C_2	1.0	00.0	0.80 L
C_3	2.0	00.0	1.00 K
C_4	3.0	00.0	1.32 J
C_5	0.0	40.5	0.45 N
C_6	1.0	40.5	1.63 I
C_7	2.0	40.5	1.84 H
C_8	3.0	40.5	2.00 G
C_9	0.0	81.0	0.53 N
C_{10}	1.0	81.0	2.26 F
C_{11}	2.0	81.0	2.65 E
C_{12}	3.0	81.0	2.83 D
C_{13}	0.0	162.0	0.65 M
C_{14}	1.0	162.0	4.67 A
C_{15}	2.0	162.0	3.20 B
C_{16}	3.0	162.0	3.00 C
$L.S.D_{0.05}$			0.109

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

The present results also partially agreed with those reported by Bondok *et al.* (1987). They found that rooting of proliferated shoots in MM106 apple rootstock, "Anna" and "Baladi" apple cultivars, was achieved on MS medium at 1/4 salt strength supplemented with activated charcoal and IBA. In addition, Shawky *et al.* (1993) mentioned that the best rooting of proliferated shoots in M26 apple rootstock could be obtained by using half strength of MS basal medium supplemented with 1.0 $mg1^{-1}$ IBA. Furthermore, Correa *et al.* (1994) stated that the best rooting (85%) of proliferated shoots in apple rootstock MI-793, was obtained with IBA at 1.0 $mg1^{-1}$ plus 100% mineral salts of MS medium.

Recently, Ferri *et al.* (1998) mentioned that IBA at 5.0 µM in half-strength Murashige and Skoog (1962) medium, produced the highest number of roots (3.5 roots / shoot explant) in apple rootstock MM111. Moreover,

Centellas *et al.* (1999) reported that NAA and IBA, both at 3.0 µM on MS/2 medium, showed similar effects in terms of rooting percentage and number of roots in apple shoots (cv. Fred Hough) derived from *in vitro* shoot multiplication. In the meantime, JunBao *et al.* (1999) found that shoots of apple cv. Fuji required 1.0 $mg1^{-1}$ IBA in MS medium for rooting. In addition, Modgil *et al.* (1999) reported that the inclusion of 100 $mg1^{-1}$ phloroglucinol (PG) in the agar-solidified MS medium proved beneficial for early and better root development of multiplicated shoots in apple cv. Tydeman's Early Worcester.

On the contrary, the results of the present study disagreed with those reported by Lisek (1996). Who found that the best rooting was obtained on Woody Plant Medium with 1.0 $mg1^{-1}$ IBA and 6.0 $g1^{-1}$ agar. He also mentioned that, phloroglucinol had a negative effect on rooting of shoots in Polish dwarf apple rootstock P59. In addition, Centellas *et al.* (1998) with apple c.v. Fred Hough shoots came to the same result with respect to the negative effect of phloroglucinol on rooting percentage or root length.

2. Transplanting of Plants to Soil

The obtained plants of MM106 apple rootstock were transferred to greenhouse conditions (after acclimatization in growth chamber). These plants demonstrated normal shape, uniformity, vigorously growing and healthy appearance (deeper green color and more expanded leaves) under the greenhouse conditions. Finally, 75% of the obtained plants of MM106 apple rootstock were successfully transplanted to soil.

These findings agreed with those obtained by Bolar *et al.* (1998). They noticed that plants of apple cultivars were transferred to pots and covered with plastic bags to facilitate acclimatization. This technique resulted in 70 to 100% of shoots selected *in vitro* producing vigorously growing and healthy plants in the greenhouse.

In addition, Isutsa *et al.* (1998) found that microshoots of apple rootstocks were acclimatized *ex vitro* in a peat: perlite (1 : 2 v/v) medium. In the same line, Modgil *et al.* (1999) mentioned that the micropropagated plants of apple cv. Tydeman's Early Worcester showed 90% survival in nursery conditions.

The present study gives a very detailed protocol for *in vitro* propagation of MM106 apple rootstock.

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الإكثار المعملى الدقيق لأصل التفاح مولنج مورتن ١٠٦
٢- التجذير والأقلمة

محمد بدر الصبروت

قسم الفاكهة – كلية الزراعة – جامعة الإسكندرية – الإسكندرية – مصر

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 نسبة إندول حمض البيوتريك إلى الفلوروجلوسينول فى بيئة الزراعة (نصف تركيز أملاح بيئة موراشيج وسكوك ، ١٩٦٢)على تجذير الأفرخ وبالتالى إنتاج النبيتات التى يجرى لها عملية أقلمة فيما بعد. ويمكن تلخيص النتائج الرئيسية لهذه الدراسة فى النقاط التالية:-

١- أدى استخدام نصف تركيز أملاح بيئة الزراعة موراشيج وسكوك (١٩٦٢) والمحتوية على ١,٠ملجرام فى اللتر إندول حمض البيوتريك + ١٦٢,٠ ملجرام فى اللتر فلوروجلوسينول إلى الحصول على قيمة مرتفعة لنسبة المئوية لتجذير الأفرخ وذلك بصورة جوهرية (١٠٠%) وكانت قيمة متوسط عدد الجذور بالنسبة للفرخ الواحد (٧,٦) وقيمة متوسط طول الجذر بالنسبة للفرخ (٤,٦٧سم) مرتفعة بصورة جوهرية.

٢- خمسة وسبعون فى المائة من النباتات المتحصل عليها لأصل التفاح مولنج مورتن ١٠٦ تم نقلها إلى التربة بنجاح وكانت هذه النباتات متماثلة وقوية النمو وسليمة تحت ظروف الصوبة.