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Abstract

This study included the effect of three-auxin types (IAA, IBA and NAA) with different concentrations each at (0.0, 0.5, 1, 2 and 4 $mg.l^{-1}$) on microshoots rooting ability. The highest mean number of roots per shoot, mean root length and rooting percentage (3.99 roots, 3.5 cm and 90%) and (3.50 roots, 3.20 cm and 90%) for both apple and pear were recorded respectively at 2 $mg.l^{-1}$ NAA. While IAA or IBA were less effective than NAA on shoot rooting ability at the same concentrations. The study also included the effect of different concentrations of MS salts strength medium on rooting ability. In apple, the best number of roots per shoot, mean root length and rooting percentage (1.95 roots, 2.23 cm and 90%) were obtained on the half strength salt of MS medium, which were significantly differed from other treatments. In pear, the highest mean number of roots per shoot and rooting percentage (1.84 roots and 80%) were obtained on the half strength salts MS medium. Finally, at the acclimatization stage, 85% of both apple and pear plantlets were survived.

Keywords: Apple rootstock (Malus domestica Borkh.) MM106, Pear rootstock (Pyrus calleryana), Auxine (IAA, IBA and NAA), MS salts strength, Shoot rooting.

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Introduction

MM106 is a clone of apple that has been originated as a cross between Northern spy and Malling 1 (Hartmann *et al.*, 2002). MM106 rootstock has been adopted in Iraq for its good characteristics (Yousif, 1987). This rootstock produces an apple tree of about 3.6 meters wide and 3 meters tall at maturity. Apple trees on MM106 rootstock can easily be pruned to keep them to a height of around 2 to 2.5 meters and they will not require staking. The roots are reasonably vigorous and an MM106 rootstock apple tree can easily support itself without the need for staking. The tree will not grow too large, so that the average person will find it easy to prune. It may be a good combination for semi-dwarfing and sometimes referred to as semi- vigorous. This is the most widely used of rootstocks. It is tolerant to a wide range of soils (Wikipedia article, 2003).

Pyrus calleryana is a blight tolerant rootstock, produces vigorous trees with a strong union area. *Pyrus calleryana* rootstock produces good fruits without black ends, however it is intolerant in areas with sever cold winters and it lacks winter hardness. It is resistant to pear decline but it has lower resistance to oak's root fungus than the French pear rootstocks (Yousif, 2002).

Pome fruits like apples and pears are the most important deciduous trees in Kurdistan region of Iraq and grown very well in the northern area of Duhok Governorate. There are many problems associated with their production like low productivity and bad quality due to cultivar decline and different infections. As with many commercial crops, pome fruits are also subjected to various biotic and abiotic stresses, since virus and viroids have been recognized as serious problem limiting the vigor, yield and quality. So, they need to be managed and developed to produce new pathogen-free orchards. Rahif *et al.* (2000) achieved the high rooting rate reached to 11.4 roots/shoot of apple MM106 rootstock when cultured on MS medium supplemented with 1 $mg.l^{-1}$ IBA. Erig and Schuch (2006) studied the effect of three auxin types (IBA, IAA and NAA) at different concentrations (0, 1, 2, 3 and 4 $mg.l^{-1}$) on rooting of quince (*Cydonia oblonga*) cv. Mc rootstock, and found that auxins (IBA, NAA, and IAA) had the same effect on rooting percentage, but rooting was greatest at 2 $mg.l^{-1}$ NAA which resulted in the greatest mean root length. Thakur and Kanwar (2008) experimented MS medium supplemented with different levels (0.125 and 0.25 $mg.l^{-1}$) of IBA and NAA for rooting of wild pear (*Pyrus prolifolia* Burm) and found that higher rooting percentage was achieved at 0.25 $mg.l^{-1}$ of NAA with 2.53 roots/explant, while IBA at 0.25 $mg.l^{-1}$ produced 1.18 roots/shoot.

Satisfactory rooting can take place on full strength culture MS medium, however, it is a very common practice to transfer shoot to be rooted from high strength medium to less concentrated solution, this practice is used for woody ornamentals, fruit trees or forestry species (George, 1996 and Monocousin, 1998). The promotory effect of a diluted mineral solution on rooting can be explained by the reduction of nitrogen concentration (Driver and Suttle, 1987 and Al-Bahir *et al.*, 1999). Banno *et al.* (1988) have studied the effect of different concentrations of salts (full, half, quarter and WPM medium) supplemented with 1 $mg.l^{-1}$ IBA in shoot rooting of Japanese pear rootstock and obtained best results when cultured on half strength which reached 100%. Dwivedi and Bist (1999) found that microcutting of pear cv. Gola was best rooted in ½ MS medium supplemented with 1.0 - 2.0 $mg.l^{-1}$ of IBA. Bader *et al*. (2000) were able to obtain high rooting (100%) when cultured shoots of pear rootstock (*Pyrus callaryana*) on half strength MS medium supplemented with 1.5 $mg.l^{-1}$ NAA, in which average root number was reached to 2,7 roots/shoot. Al-Chalabi *et al*. (2003) studied the effect of different concentrations of salts (full, half and quarter) supplemented with different levels of IAA and IBA on rooting of apple, they found that quarter strength MS medium with 10 $mg.l^{-1}$ IAA produced best rooting ability (100% rooting, 40 cm length and 15 roots/ shoot).The present study was carried out to determine the effects of different Auxin concentrations and the effect of MS salts on *in vitro* root production of both apple and pear rootstocks.

Materials and Methods

The explants employed were shoots of MM106 apple rootstock (*Malus domestica*) and pear rootstock (*Pyrus calleryana*) of about 1cm in length, preserved from previous *in vitro* cultures and maintained in the growth room. Each explant was transferred and grown in Mason jar containing 25 ml of MS culture medium (Murashige and Skoog, 1962) and capped with polyethylene covers. Two experiments were conducted, in the first one, the effect of different auxin type and concentrations (IAA, IBA and NAA) each at the same concentrations (0, 0.5, 1, 2, 4 $mg.l^{-1}$) on the rooting of both apple and pear rootstocks was studied. A total of 5 treatments were employed with 10 replications per treatment. In the second experiment, the effect of inorganic slats concentrations of a MS medium (full strength, half strength, quarter strength and double strength) supplemented with IBA at 1$mg.l^{-1}$ on the rooting performance of apple and pear rootstock was investigated. A total of 4 treatments were employed with 10 replications per treatment. The pH of the medium was adjusted to 5.7 and autoclaved at 121ºC for 20 min. All the cultures were incubated at 25 ± 2ºC and cultures were kept under a 16 h photoperiod fluorescent tube light. The statistical design employed was the complete randomized design (C.R.D). Significant differences among means values were separated and compared using Duncan's multiple range tests at 5% level. All statistical analysis were performed using Statical Analysis System (SAS, 2001). After 6 weeks from shoots rooting, the plantlets were thoroughly washed with tap water to remove agar from the roots, which might be a source of contamination. The plantlets were put in Benlate fungicide solution (0.1%) for 10 minutes before planted in plastic pots (5 cm diameter) containing autoclaved soil mixture of peatmoss, loam and Styrofoam in a ratio of (1:1:0.5, v:v:v) covered with transparent poly ethylene to maintain high illumination and relative humidity. The potted plants were placed in incubation room for 14 days. The plants were irrigated with quarter (0.25X) salt strength of MS salts according to their need, and they were gradually acclimatized by progressively opening the cover until they were ready to transfer to the green house.

Results and Discussion

Table (1) illustrates the effect of different concentrations of IAA, IBA and NAA on rooting ability of apple microcuttings. The highest number of roots per shoots (1.55) and the highest rooting percent (40 %) were observed on the medium supplemented with 2 $mg.l^{-1}$ IAA, while the highest mean length of roots (2.10 cm) was recorded at 1 $mg.l^{-1}$ IAA level. Roots number was increased with increasing IAA concentrations until 2 $mg.l^{-1}$ then decreased when IAA concentration was increased. The roots were initiated following 2-3 weeks of incubation on rooting medium. Roots were adventitiously initiated at cut margins of the shoots. One of the major physiological effects of the auxins is stimulation of adventitious roots formation in both *in vitro* and *in vivo* cuttings (Hartmann *et al*., 2002).

Addition of 2 $mg.l^{-1}$ of IBA was superior upon the rest treatments in producing highest root number per shoots (2.85) and highest rooting percent (80%) and the longest root mean estimated at 2.30 cm. While the lowest number of roots per shoot (0.94), least mean length of roots (0.76 cm) and the least rooting percent (4%) were recorded on auxin-free medium (control).

The highest number of roots per shoots (3.99), the longest roots mean (3.50 cm) and the highest rooting percent (90%) were observed on MS medium supplemented with 2 $mg.l^{-1}$ NAA, which was significantly higher than the rest treatments.

The effect of different concentrations of IAA, IBA and NAA on rooting ability of pear microcuttings is shown in Table (2). The 2 $mg.l^{-1}$ IAA level gave the highest roots number, roots mean length and rooting percent (1.07 roots/ shoots, 1.67 cm and 30%) respectively. The roots were initiated following 2-3 weeks of incubation on rooting medium. Roots were adventitiously initiated at shoots margins of the shoots. Similar observations were reported by Welander andSnygg (1987) and Shibli *et al*. (1997).

The highest number of roots per shoots (2.54), the longest roots mean (1.80 cm) and the highest rooting percent (80%) were observed on the medium supplemented with 1 $mg.l^{-1}$ IBA. These observations may indicate that IBA showed better performance on rooting than IAA. Such performance could be attributed to the higher stability of IBA when compared to IAA, which is light-labile compound (George *et al.*, 2008). In addition, there are many differences in metabolism and transport of both auxins, which affect their response or physiological effects on adventitious root formation (Epstein and Miller, 1993). Results attained in this investigation are consistent with those reported by Tantos (2002); Sedlak and Peperstein (2003) and Al-Ansary *et al*. (2007).

High root number (3.50), high root length (3.20 cm) and high rooting percentage (90%) were obtained at 2 $mg.l^{-1}$ of NAA. The mean of root number and root length at 2 $mg.l^{-1}$ NAA were significantly different when compared with other concentrations. NAA at high concentration (4 $mg.l^{-1}$) decreased root initiation and elongation. This result agree with those of Hartmann *et al*. (2002) when they noticed that high NAA concentrations influenced rooting negatively since auxin stimulates root initiation but inhibits root elongation. Apple and pear produced high rooting number, root length and rooting percentage at 2 $mg.l^{-1}$ of NAA and these results agree with those reported by Erig and Schuch (2006); Al-Ansary *et al*. (2007) and Cosac *et al*. (2008). These results also proved that NAA is a more active auxin than IAA and IBA and can be used at a lower concentration (Lane, 1992). Fouad *et al*. (1997) have reported that the most widely used method for *in vitro* rooting of many woody plant species is based on the use of different types and concentrations of auxins in rooting medium.

In conclusion, rooting results obtained in this investigation confirmed the need of auxins for apple and pear adventitious root formation. These results indicated that the presence of auxins have positive influences on rhizogenesis in apple and pear *in vitro*. In addition, the most effective auxin in rooting of apple and pear was NAA followed by IBA and IAA. Such differences in potency of auxin in inducing rooting may be attributed to the structure of the auxins under study, endogenous hormone level, as well as genetic makeup of species under consideration. This also explains the different response of different species based on these variations (Karhu and Zimmerman, 1993).

The physiological effects of auxins are represented in increasing of cell division or converting the matured differentiated cells in shoots bases into meristematic cells (totipotent cells), so adventitious roots meristem will be formed and its cells will divide to produce adventitious roots (Abdul, 1987; Saleh, 1991 and Hartmann *et al*., 2002). Endogenous hormones might have a role in promoting shoots or roots (Peak *et al*., 1987), until the hormonal balance reached its optimal level to push the roots to grow and develop in the presence of exogenous hormones, since increasing of auxins concentration promotes root formation on shoots bases (George and Sherrington, 1984). The mechanism of action of such concentration of each auxin may be explained based on endogenous levels of the auxin under consideration. Inclusion of exogenous auxin in the culture medium will alter the endogenous level, which may trigger the process of adventitious root initiation and subsequent development. In addition, the differences in the genetic makeup of various varieties may also play a role in this regard (Tichoux, 1999).

Effect of MS salts concentration on rooting ability of microcuttings

Effects of different inorganic salt concentrations (quarter, half, full and double salt strengths) were investigated in rooting ability of apple and pear. Table (3) illustrates the effect of different MS salts concentrations on apple and pear rooting ability. Regarding apple, results showed that highest root number (1.95), maximum root length (2.23 cm) and high root percent (90%) were observed on half salt strength MS medium which was significantly different from those on full and double strength MS medium, however, did not showed significant difference from quarter strength MS medium.

Regarding pear, half strength MS medium was also superior when compared to other treatments, which produced highest root number per explants (1.84) and highest rooting percent (80%). This was significantly different from full and double strength MS medium, however, did not showed significant difference from quarter strength MS medium. On the other hand, the longest root mean was obtained at quarter salt strength, which estimated as 1.93 cm and according to this table, the half-salt strength MS medium was the optimum salt strength when compared with other salt strength. This may be due to the increase of carbohydrate ratio to nitrogen, which mean increasing energy source (carbohydrate) which considered necessary for rooting (Hartmann *et al*., 2002).

The promoting effect of mineral concentration of the culture medium on rooting can be attained as inorganic ions, which participate in the process of regulating hormonal balance (Amzallag *et al*., 1992). Satisfactory rooting can take place in full strength culture medium, but is a very common practice to transfer the shoots to be rooted from high strength media to less concentrated solution. This practice is used for herbaceous plants as well as woody ornamentals, fruit trees and forest species (Monocousin, 1998). The favorable effect of a diluted mineral solution on rooting can be explained by the reduction of nitrogen concentration (Driver and Suttle, 1987 and Al-Bahir *et al*., 1999). The mineral concentration in the culture medium affects rooting characteristic and some researchers have proposed that reduction of salt strength to half strength improved rooting (Dimassi-Theriou and Economou, 1993). The reason behind increasing rooting rate on half strength culture medium is might be back to a disorder in carbohydrate to nitrogen in nutrient medium, which lead to decreasing nitrogen level in shoot and then improving rooting rate, initiation roots, increasing root number and lengths (Salman, 1988). These results in both apple and pear rooting are similar with those reported by Dwivedi and Bist (1999); Al-Chalabi *et al*. (2002) and Lucyszyn *et al*. (2006).

Acclimatization Stage

This stage is the most important in micropropagation which the success or failure of the *in vitro* experiments depends on. After 6 weeks in rooting medium, the rooted plantlets were washed under tap water to remove the residues of culture medium, which is a goal of microorganism's attacks because of its sugar and agar content. It is preferred to emerge the plants into a fungicide solution (Benlate, 0.1% for 10 minutes) to protect them from fungal attacks, and then transferred to pots containing a steam sterilized soil mixture (peatmoss + loam + styrofoam 1:1: 0.5, v: v: v). Pots were enclosed with polyethylene bags, which were closed and placed in growth room at the lab. in which the temperature was set at 23-25ºC and exposed daily to 16 hr 1000 lux illumination. The plants were irrigated with a nutrient solution containing 1/4 strength of MS salts. After a week, the polyethylene bags were pored, and after 10 days, the bags were opened and then after 15 days, the bags were removed and plants were grown under regular greenhouse conditions.

Successful gradually acclimatization of apple and pear reached 85% and after then plantlets grew well and did not show morphological abnormalities. These steps of vegetative micropropagation agree with what has been found by many other researches in fruit plants which later moved to open air field (Caboni *et al.*, 2000; Sedlak and Paprstein, 2003; Banno *et al.*, 2007 and Yildirim *et al.*, 2007).

Conclusions

From the achieved results, it can be concluded that the optimum auxin for the rooting of apple and pear microcuttings was NAA at 2 mg.l^{-1}, which gave better rooting parameters as compared with the use of IAA or IBA and a good rooting parameters were obtained while culturing microcuttings on half strength MS medium supplemented with 1 mg.l^{-1} IBA.

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Table 1. *Effect of different concentrations of IAA,IBA and NAA on rooting ability of apple microcuttings cultured on MS medium after 6 weeks of culture*

Type of Auxins	Different concentrations ($mg.l^{-1}$)	Percent of shoots rooted (%)	Number of roots/shoot (Mean ± S.E.)	Mean length of roots (cm) (Mean ± S.E.)
IAA	0.0	5 b*	0.25 ± 0.02 b	0.43 ± 0.03 b
	0.5	10 ab	0.74 ± 0.08 b	0.98 ± 0.17 b
	1.0	20 ab	1.06 ± 0.18 b	2.10 ± 0.23 a
	2.0	40 a	1.55 ± 0.28 a	1.35 ± 0.19 a
	4.0	30 a	1.45 ± 0.17 a	1.45 ± 0.19 a
IBA	0.0	4 c*	0.94 ± 0.09 b	0.76 ± 0.07 b
	0.5	10 c	1.04 ± 0.10 b	1.07 ± 0.20 ab
	1.0	45 b	1.56 ± 0.14 b	1.56 ± 0.14 a
	2.0	80 a	2.85 ± 0.49 a	2.30 ± 0.14 a
	4.0	60 a	2.34 ± 0.26 a	1.30 ± 0.13 ab
NAA	0.0	3 c*	1.00 ± 0.18 c	1.06 ± 0.12 c
	0.5	30 b	1.50 ± 0.21 c	1.20 ± 0.22 c
	1.0	80 a	2.50 ± 0.34 b	2.39 ± 0.26 b
	2.0	90 a	3.99 ± 0.88 a	3.50 ± 0.45 a
	4.0	80 a	2.30 ± 0.46 b	2.10 ± 0.33 b

***Means with the same letters are not significantly different according to Duncan's multiple range tests at $P \leq 0.05$.**

Table 2. Effect of different concentrations of IAA, IBA and NAA on rooting ability of pear microcuttings cultured on MS medium after 6 weeks of culture.

Type of Auxins	Different concentrations (mg.l^{-1})	Percent of shoots rooted (%)	Number of roots/shoot (Mean ± S.E.)	Mean length of roots (cm) (Mean ± S.E.)
IAA	0.0	5 c*	0.35 ± 0.05 b	0.56 ± 0.07 b
	0.5	15 b	0.73 ± 0.07 ab	0.79 ± 0.04 b
	1.0	20 ab	0.74 ± 0.15 ab	0.87 ± 0.07 b
	2.0	30 a	1.07 ± 0.17 a	1.67 ± 0.20 a
	4.0	20 ab	0.95 ± 0.07 ab	1.30 ± 0.14 a
IBA	0.0	6 c*	0.87 ± 0.05 b	0.58 ± 0.04 b
	0.5	10 bc	1.03 ± 0.14 b	1.00 ± 0.16 b
	1.0	80 a	2.54 ± 0.27 a	1.80 ± 0.21 a
	2.0	50 b	2.07 ± 0.23 a	1.65 ± 0.18 a
	4.0	30 b	1.87 ± 0.19 a	1.45 ± 0.15 a
NAA	0.0	5 b*	0.75 ± 0.15 c	0.57 ± 0.12 c
	0.5	60 a	1.05 ± 0.39 c	1.76 ± 0.28 b
	1.0	70 a	2.00 ± 0.41 b	2.00 ± 0.29 b
	2.0	90 a	3.50 ± 0.84 a	3.20 ± 0.45 a
	4.0	80 a	2.45 ± 0.43 b	2.27 ± 0.31 b

***Means with the same letters are not significantly different according to Duncan's multiple range tests at P ≤ 0.05.**

Table 3. Effect of different MS salts concentrations on rooting ability of apple and pear microcuttings cultured on MS medium supplemented with 1mg.l^{-1} IBA after 6 weeks of culture.

Apple / Pear	MS Salts concentration	Percent of shoots rooted (%)	Number of roots/shoot (Mean ± S.E.)	Mean length of roots (cm) (Mean ± S.E.)
Apple	0.25	75 a*	1.78 ± 0.28 a	2.00 ± 0.40 a
	0.5	90 a	1.95 ± 0.20 a	2.23 ± 0.33 a
	1	40 b	1.14 ± 0.03 b	1.13 ± 0.27 b
	2	5 c	0.49 ± 0.04 c	0.57 ± 0.02 c
Pear	0.25	60 a*	1.53 ± 0.32 a	1.93 ± 0.42 a
	0.5	80 a	1.84 ± 0.24 a	1.34 ± 0.23 a
	1	30 b	1.25 ± 0.14 b	0.84 ± 0.17 b
	2	6 c	0.45 ± 0.10 c	0.55 ± 0.02 b

***Means with the same letters are not significantly different according to Duncan's multiple range tests at P ≤ 0.05.**