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IN VITRO PROPAGATION OF TWO CITRUS ROOTSTOCKS BY SHOOT TIP CULTURE III. ROOTING STUDY

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ABSTRACT: Multiplicated shoots of sour orange and volkamer lemon rootstocks longer than (1.5 cm) were selected for rooting stage, which were cultured on some tested rooting media. Basal media (M.S medium) were supplemented with NAA at (0.0 control, 0.5, 1.0, 2.0 and 5.0 mg/l) or IBA at (0.0 control and 5.0 mg/l) and IAA at (0.0 control and 2.0 mg/l), beside various combination between NAA, IBA and IAA. Multiplicated shoots cultured on M. S. medium only (control) and lacking growth regulators failed to give any roots in both tested rootstocks. However, M.S. medium supplemented with 5 mg/l NAA (in sour orange) and 5mg/l IBA (in volkamer lemon) gave the highest percentage of sprouted roots (48.25%) and (51.32%), respectively. In addition, root numbers / shoot reached to the maximum in multiplicated shoots of sour orange cultured on M.S + 5 mg/l NAA and M.S. + 5mg/l IBA for volkamer lemon and the other values ranged between control treatment and the above mentioned treatments. Explants taken in March significantly increased rooting (%), root numbers / shoot and root length as compared with December samples under different tested media for both evaluated rootstocks. Thickness of formed roots in both rootstocks significantly affected in response to type and concentration of applied growth regulators, in addition to date of sampling. Explants gathering in December significantly increased root thickness than those taken in March under different studied media for both examined rootstocks.

The obtained data also show that, agar application to M.S. medium supplemented with GR improved root growth and development and semi-solid medium (M.S.+4g agar/l) was considered the most proper medium for both tested rootstocks as compared with solid (6.g agar/l) or liquid (without agar) media. This is true in both tested rootstocks.

Keywords: Citrus rootstocks; growth regulators (GR); agar; nutrient medium (M.S.); multiplicated shoots and roots formation.

INTRODUCTION

Micropropagation uses the technique of plant tissue culture (as avegetative way to improve the laboratory production and to increase rapid clonal multiplication rate of both tested rotstocks) and explants of shoot tips are the most successful and the simplest micropropagation system. *In vitro* roots formation clearly affected by many factors such as: plant sp., type and concentration of GR added to the media, beside date of explant gathering (Stino *et al.* 1980; Fanizza *et al.*, 1981; Pasqual and Ando 1990; Hb songlin *et al.*, 1996; Badr, 1998; Singh *et al.*, 1999 and Matsumoto *et al.* , 2000).

Accordingly, characters of formed roots such as : rooting (%), root numbers/ shoot, length and thickness of sprouted roots clearly affected by many factors (Kitto and Young, 1981; Lukman *et al.*, 1990; Lin *et al.*, 1992; Mass *et al.* 1994; Ribeiro *et al.*, 1999 and Moreira - Dias *et al.*, 2000). In this concern, root characters significantly, affected in their response to agar requirements when applied to basal nutrient medium (Pasqual *et al.*, 1985 and Ribeiro *et al.*, 1999).

The main objective of this experiment is to study the effect of some growth regulators such as: NAA, IBA and IAA and agar substitutes applied to M.S. medium on roots formation and development of multiplicated shoots (Part II) in sour orange and volkamer lemon rootstocks, in addition to the residual effect of sampling date (active and inactive growth phases) through starting and multiplication stages of this study.

MATERIALS AND METHODS

This investigation was carried out in tissue culture laboratory of Horticulture Department, Agriculture Faculty of Zagazig University during (2001 and 2002, seasons). Two rootstocks were used namely: Sour orange (*Citrus aurantium* L.) and volkamer lemon (*Citrus volkameriana* Ten.). Explants of both tested rootstocks were excised from mother trees (with 0.5 and 1 cm in long) in two periods (time), the first in March (active phase) and the second in December (dormant phase). *In vitro*, explants of both studied rootstocks were prepared through different treatments (Part 1 and 2) of this study then passed through stages of shoot tip culture *in vitro* (starting

and multiplication) as mentioned in the first part (starting stage) and second part (multiplication stage) of this study.

Healthy adventitious (multiplicated) shoots in their appearance produced from explants (shoot tip) regeneration *in vitro* were selected for rooting study. As such, multiplicated shoots longer than (1.5cm) obtained from multiplication stage were cultured on different tested rooting media consisted of basal M.S. medium supplemented with NAA at concentrations of (0.0, 0.5, 1.0, 2.0 and 5.0 mg/l), IBA at concentrations of (0.0 and 5.0 mg/l and IAA at concentrations of (0.0 and 2.0 mg/l), beside various combinations between NAA, IBA and IAA (2.0 mg/l NAA+5.0 mg/IBA); (2.0 mg/l NAA + 2mg/l IAA) and (2.0 NAA + 5.0 IBA + 2 IAA as mg/l) were used. In this concern, many investigators (Kitto and Young, 1981; Starrantino and Caruse, 1988; Marine and Duran-Vila, 1992; Matsumoto *et al.*, 1998 and El-Wakeel, 1999) used the above mentioned GR in rooting citrus species and hybrids and other fruit species. All tested rooting media were supplemented also with 45g/l sucrose.

As for agar, three concentrations of agar were used as follows: (6 g/l) to obtain a solid medium, (4 g/l) for semi- solid medium and liquid medium were obtained from without agar application and used as check (control) treatment. All culture media incubated at (26±2°C) and (8/16 hrs. day /night) using fluorescent lamps (40w) approx. induced 2000 Lux.

Explants were repeatedly subcultured after 5 weeks of *in vitro* culture on corresponding and fresh M.S medium supplemented with 2.0 mg/l NAA and 5.0 mg/l IBA for sour orange and volkamer lemon , respectively in March and December, beside 45 g/l sucrose and 4 g/l agar.

The obtained data were taken after 5 weeks of *in vitro* culture on different tested rooting media, rooting (%) number, length (cm) and thickness (mm) of sprouted roots formed on multiplicated shoots and finally shoot/ root ratio of total plantlets length.

Data were subjected to analysis by using the method of Snedecor and Cochran (1980). Meanwhile, data calculated in percentage were transformed to angles (angle = ARG Sin $\sqrt{percentage}$ before

analysis. The individual comparisons were carried out by using Duncan's multiple range test at (0.05 level).

RESULTS AND DISCUSSION

Rooting stage included two main separated experiments, the first one was concerned with the effect of M.S. medium (control) and M.S. medium supplemented with some growth regulators (GR) such as (NAA, IBA and IAA) at different concentrations, all tested and control treatments were supplemented beside growth regulators with (6 g/l) agar. However, the second experiment concerning the effect of agar application to M.S. medium such as : liquid medium (without agar), (4 g agar/l) to form semi-solid medium and (6 g agar/l) to obtain solid medium.

First Experiment:

Percentage of roots formation:

As shown in (Tables 1, 2) percentage of formed roots on cultured multiplicated shoots significantly varied between (0.00 and 48.25%) and (0.0 and 52.63%) for sour orange and volkamer lemon rootstocks, respectively, increasing NAA concentration in M.S medium tended to increase rooting (%) with values reached to (32.07, 36.37, 46.32 and 48.25%) in sour orange rootstock and (29.25, 34.07, 36.10 and 43.00%) in volkamer lemon rootstock for M.S medium contained NAA at (0.5, 1.0, 2.0 and 5.0 mg/l) respectively.

IBA added to different tested rooting media increased rooting (%) than M.S medium lacking growth regulators for both examined rootstocks. Concerning the effect of sampling date, explants gathering in March was superior as compared with those taken in December (42.83 against 30.28%) and (46.23 against 29.35%) for sour orange and volkamer lemon rootstocks, respectively.

It can be concluded that, M.S medium supplemented with 2.0 or 5.0 mg/l NAA was the best as compared with the control or other tested ones.

Interaction was statistically significant and acted dependently.

Root number per shoot:

As noticed in (Tables 1, 2) root numbers/ shoot significantly differed in their response to type and concentration of growth regulators. As such, the highest values of root numbers/ shoot

Table 1: Effect of some growth regulators added to M. S medium (mg/l) on rooting of sour orange rootstock (2001/2002, seasons).

Treat. NAA, IBA, IAA (mg/l)			Root formation (%)			Root number /shoot			Root length (cm)			Root thickness (mm)		
Control			March	Dec.	Mean	March	Dec.	Mean	March	Dec.	Mean	March	Dec.	Mean
(M. S medium only)			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	-	-	44.05	20.10	32.07	2.50	2.00	2.25	1.55	1.00	1.27	0.72	0.60	0.66
1.0	-	-	48.75	24.00	36.37	2.50	2.55	2.52	1.95	1.00	1.47	0.72	0.80	0.76
2.0	-	-	52.50	40.15	46.32	3.00	3.00	3.00	3.00	2.50	2.75	0.66	0.77	0.71
5.0	-	-	52.00	44.50	48.25	5.50	3.50	4.50	2.00	1.75	1.87	0.68	0.70	0.69
-	5.0	-	40.15	28.80	34.47	2.50	2.05	2.27	2.20	1.20	1.70	0.62	0.69	0.65
2.0	-	2.0	52.10	40.50	46.30	3.50	3.11	3.30	3.00	2.00	2.50	0.50	0.70	0.60
2.0	5.0	-	44.50	36.00	40.25	3.50	2.50	3.00	2.70	2.00	2.35	0.60	0.71	0.65
2.0	5.0	2.0	51.50	38.50	45.00	5.25	3.10	4.17	2.00	1.00	1.50	0.52	0.50	0.51
Mean			42.83	30.28			2.42		2.04	1.38		0.55	0.60	-
New LSD														
D (Date):				(4.575)			(0.236)			(0.155)			(N.S)	
C (concentration)				(3.603)			(0.236)			(0.219)			(0.22)	
Interaction:														
D x C:				(7.432)			(0.289)			(0.219)			(0.18)	

Table 2: Effect of some growth regulators added to M. S medium (mg/l) on rooting of volkamer lemon rootstock (2001/2002, seasons).

Treat. NAA, IBA, IAA (mg/l)			Root formation (%)			Root number /shoot			Root length (cm)			Root thickness (mm)		
Control			March	Dec.	Mean	March	Dec.	Mean	March	Dec.	Mean	March	Dec.	Mean
(M. S medium only)			0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.5	-	-	40.50	18.00	29.25	3.10	2.00	2.55	1.50	2.05	1.77	0.70	0.60	0.65
1.0	-	-	44.05	24.10	34.07	3.40	2.20	2.80	1.75	2.10	1.92	0.70	0.60	0.65
2.0	-	-	46.60	25.60	36.10	3.50	2.25	2.87	2.20	1.50	1.85	0.66	0.72	0.69
5.0	-	-	50.50	35.50	43.00	4.40	3.25	3.82	2.35	1.75	2.05	0.59	0.72	0.65
-	5.0	-	62.10	40.55	51.32	5.55	4.75	5.15	3.55	3.35	3.45	0.65	0.70	0.67
2.0	-	2.0	52.17	35.00	43.58	4.45	3.05	3.75	2.55	2.30	2.42	0.72	0.73	0.72
2.0	5.0	-	60.01	40.40	50.20	4.50	4.20	4.35	3.38	2.50	2.81	0.67	0.70	0.68
2.0	5.0	2.0	60.22	45.05	52.63	4.55	4.50	4.52	2.12	1.15	1.63	0.52	0.60	0.56
Mean			46.23	29.35		3.71	2.91		2.15	1.82		0.57	0.59	

New LSD				
D (Date):	(3.575)	(0.236)	(0.126)	(N.S)
C (concentration)	(2.603)	(0.289)	(0.126)	(0.22)
Interaction:				
D x C:	(7.421)	(0.236)	(0.179)	(N.S)

produced in M.S medium contained 5 mg/l NAA (4.5 roots / shoot) in sour orange rootstock; while, in volkamer lemon rootstock, IBA at 5mg/l added to M.S medium solely increased number of formed roots (5.15 roots / shoot) as compared with other tested treatments and considered the best for rooting either solely or combined with NAA and IAA.

Concerning the effect of sampling date, March sampling was superior than December one (3.13 against 2.42 roots / shoot) in sour orange rootstock and (3.71 against 2.91 roots / shoot) in volkamer lemon rootstock, respectively; while, multiplicated shoots cultured on M.S medium lacking growth regulators failed to give any roots. This is clear in both examined rootstocks.

Interaction study concerning root numbers / shoot was statistically significant in most cases.

Root length:

From (Tables1,2),multiplicated shoots cultured on M.S medium contained 2 mg/l NAA gave the longest formed roots (2.75 cm) in sour orange and on M.S. medium supplemented with 5 mg/l IBA in volkamer lemon (3.45cm). However, the shortest roots (1.27cm) in sour orange and (1.63cm) in volkamer lemon were obtained from shoots cultured on M.S. medium contained (0.5 mg/l NAA and 2 NAA + 5 IBA + 2 IAA as mg/l) in both evaluated rootstocks, respectively. Also, cultured shoots on M.S medium only (control) failed to give any roots. In this concern, root length significantly affected by sampling date and March sampling gave roots longer than December one (2.04 against 1.38cm) and (2.15 against 1.82 cm) for sour orange and volkamer lemon rootstocks, respectively.

Interaction study concerning root length was statistically significant in most cases. This is true in both examined rootstocks.

Root thickness:

M.S medium supplemented with NAA at 1 mg/l gave the thickest formed roots (0.76 mm);while, M.S medium with (2 NAA + 5IBA + 2 IAA as mg/l) gave the thinnest roots (0.51mm) in sour orange rootstock. However, significant differences could be noticed due to M.S medium consisted of different tested growth regulators and M.S medium supplemented with (2 NAA + 2 IAA as mg/l) gave the

thickest sprouted roots (0.72 mm); while, the thinnest roots (0.56 mm) were obtained from M. S. medium with (2 NAA + 5IBA + 2 IAA as mg/l). This found in volkamer lemon rootstock.

As for the effect of sampling date, explants gathering in December significantly increased thickness of formed roots than those taken in March (0.60 against 0.55mm) and (0.57 against 0.59 mm) for sour orange and volkamer lemon rootstocks, respectively.

Interaction concerning root thickness was significant in sour orange and insignificant in volkamer lemon (Tables 1, 2).

The obtained herein results are in accordance with the results of many investigators (Barlass and Skene, 1982; Starrantino and Caruse, 1988; Duran-Vila *et al.*, 1989; Pasqual and Ando ,1990; Lin *et al.*, 1992; Matsumoto *et al.*, 2000 and Moreira-Dias *et al.*, 2000) working on citrus species and hybrids. However, Lukman *et al.* (1990) reported that, NAA did not appear to promote rooting, but approx. 17% rooting occurred when the shoots were cultured in half–strength M.S medium with no NAA (M.S with NAA+IBA was superior than M.S+IBA).

Pasqual and Ando (1990), Eugenio *et al.* (1997); Matsumoto *et al.* (1998) and Nagao *et al.* (1998) came to the same conclusion and results.

In agreement with the obtained results many investigators reported that, rooting of multiplicated shoots produced from sour orange segments were optimal in M.S medium containing 1.0 mg/l IBA and 1.0 mg/l NAA (Can *et al.*, 1992). In the same direction, El-Wakeel (1999) found that, shoots taken from third, fourth, fifth and sixth multiplication subcultures were cultured on half–strength M.S medium with no growth regulators or with 1.0 mg/l IBA +1.0 mg/l NAA or 0.5 mg/l IBA +0.5 mg/l NAA. Rootstocks studied were sour orange, volkamer lemon, Balady lime and trifoliate orange. Rooting (%) was highest with 0.5 mg/l IBA + 0.5 mg/l NAA and sour orange was, generally, the poorest rooters. Also, Moreira–Dias *et al.* (2000) found that, rooting was best in the presence of 2.2 µm NAA and 2.6 µm IAA in citrange rootstock.

As for the effect of sampling date on rooting (%), it is clearly in both studied rootstocks explants gathering in March was superior, in general, as compared with those

taken in December in most cases under the same tested media. The available literature in this concern proved that the optimum time to gather *Prunms mariana* shoot tips and buds for explants was between May (25) and August (14) to insure shoot and root formation (Borred, 1971). Moreover, wood from *Castonea sativa* formed callus, the best in March and worst in December, interestingly another chestnut clone was found to grow best in March, but worst in July (Monsion and Dunez, 1971). However, Hb Songlin *et al.* (1996) proved that, shoot tips taken in July and August were least successful for tissue culture in some apple cultivars.

The obtained results also show that, M.S medium supplemented with 2.0 mg/l NAA+ 5.0mg/l IBA + 2.0 mg/l IAA increased number of formed roots/ shoot and considered good medium for roots formation and came after M.S. medium with 5.0 mg/l NAA and IBA which gave the highest number of roots/shoot and March sampling was superior than December one in this concern. The available literature concerning the effect of NAA, IBA and IAA on number of roots/ shoot are very scarce. This is true in both evaluated rootstocks.

Concerning length of roots formed on cultured shoots, M.S medium with 2.0 mg/l NAA gave the longest roots of sour orange; while M.S.+ 5 mg/l IBA gave the longest roots of volkamer lemon. Furthermore, explants taken in March gave roots longer than those taken in December in most cases under different tested media. The available literature is very rare in this concern. Singh *et al.* (1994) found that, root elongation of citremon 1452 occurred in M.S medium with 25g/l sucrose, 0.2mg/l thiamin and 0.15 mg/l NAA. In addition, Eugenio *et al.* (1997) proved that, adventitious roots among the four tested treatments ranged in average (2.9–10 roots) were produced by each shoot and the length of formed roots fluctuated between (10-40mm). Roots formed in 70% of the treated shoots.

From the obtained results, root thickness showed true differences in response to tested growth regulators in both tested rootstocks. Accordingly, M.S medium with 1.0 mg/l NAA gave the thickest roots in sour orange; while, M.S medium with 2.0 NAA + 5.0 IBA + 2.0 IAA (mg/l) increased thickness of volkamer lemon formed roots and explants taken in December

significantly also increased thickness of formed roots than those taken in March. M.S medium lacking growth regulators failed to give any roots. This is clear in both studied rootstocks under different tested treatments. The literature concerning root thickness was not available.

Second Experiment:
Percentage of roots formation:

Recorded data in (Tables 3,4) show that, rooting (%) was clearly affected by agar in rooting media and date of explant gathering. As such, M.S medium contained 4 g agar/l or (semi-solid medium) gave the highest percentage of rooting (35.50 and 39.38%) followed by M.S medium supplemented with 6g agar/l (solid medium) with percentages of 25.35 and 26.35%. However, liquid M.S medium (control) gave the lowest values (18.62 and 19.25%) in descending order for both tested rootstocks (sour orange and volkamer lemon), respectively. As for the effect of sampling date, explants of March gave the highest rooting percentage (30.36 and 35.5%) and were superior in this concern than December ones (22.61 and 21.15%) for sour orange and volkamer lemon rootstocks, respectively.

Significant differences could be noticed among different tested media concerning rooting (%). M.S. medium lacking growth regulators failed to give any roots; while, M.S medium lacking agar formed roots on culture multiplicated shoots (18.62) and (19.25%) in sour orange and volkamer lemon root stocks, respectively.

Conclusive, application of growth regulators to basal nutrient medium (M.S) was more effective than agar to promote process of roots formation. Meanwhile, agar application to M.S. medium gave better results than liquid medium. The role of agar is based on the well-recognized functions of cultured shoots by maintenance of medium in good structure and consequently improved formation of roots.

Interaction was statistically significant in this concern.

Root number per shoot:

Data in (Tables 3,4) concerning number of formed roots / shoot of cultured shoots on agar applied in M.S rooting media show that, semi-solid medium gave the highest number of roots /shoot (4.05) as compared with liquid medium (control) (2.25 and 2.00 roots/

Table 3: Effect of agar application to M.S medium on rooting of sour orange rootstock (2001/2002, seasons).

Treatments	Root formation (%)			Root number /shoot			Root length (cm)			Root thickness (mm)			Shoot / root ratio (%)		
	March	Dec.	Mean	March	Dec.	Mean	March	Dec.	Mean	March	Dec.	Mean	March	Dec.	Mean
Liquid M.S. (*)	20.25	17.00	18.62	2.50	2.00	2.25	2.60	1.50	2.05	0.55	0.75	0.65	60.50	75.00	67.75
Semi-solidM.S.(**)	40.70	30.30	35.50	4.85	3.25	4.05	3.55	2.70	3.12	0.50	1.00	0.75	40.00	50.55	45.27
Solid M.S.(***)	30.15	20.55	25.35	1..95	1.55	1.75	1.75	1.00	1.37	1.20	0.70	0.95	60.00	50.00	55.00
Mean	30.36	22.61		3.10	2.26		2.63	1.73		0.75	0.81		53.50	58.51	

(*) Control (without agar) (**) 4 g/l agar (***) 6 g/l agar

New LSD					
D (Date):	..807	0.184	0.77	0.13	3.796
C (concentration)	8.077	0.391	0.163	0.13	3.332
Interaction:					
D x C:	3.554	0.231	0.230	0.27	8.033

Table 4: Effect of agar application to M.S medium on rooting of volkamer lemon rootstock (2001/2002, seasons).

Treatments	Root formation (%)			Root number /shoot			Root length (cm)			Root thickness (mm)			Shoot / root ratio (%)		
	March	Dec.	Mean	March	Dec.	Mean	March	Dec.	Mean	March	Dec.	Mean	March	Dec.	Mean
Liquid M.S. (*)	20.35	18.15	19.25	2.00	2.00	2.00	2.75	2.00	2.37	0.50	0.75	0.62	50.00	60.60	55.30
Semi-solid M.S(**)	55.65	23.12	39.38	4.55	3.55	4.05	4.53	3.00	3.76	0.85	1.00	0.92	25.75	40.40	33.07
Solid M.S.(***)	30.50	22.20	26.35	2.75	2.50	2.62	3.42	2.50	2.96	1.00	1.00	1.00	40.45	55.55	48.00
Mean	35.50	21.15		3.10	2.68		3.56	2.50		0.78	0.91		38.73	52.18	

(*) Control (without agar) (**) 4 g/l agar (***) 6 g/l agar

New LSD					
D (Date):	3.29	N.S	0.077	0.13	3.789
C (concentration)	7.033	0.390	0.163	0.13	3.348
Interaction:					
D x C:	4.124	0.234	0.231	0.27	6.065

shoot) or solid medium (1.75 and 2.62 roots /shoot). In addition, date of sampling showed true significant differences, and March sampling increased number of formed roots/ shoot than December one (3.10 against 2.26) and (3.10 against 2.68 roots/shoot), for sour orange and volkamer lemon rootstocks, respectively. This is clear under different tested treatments.

Interaction study concerning root numbers/ shoot was statistically significant.

Root length:

The obtained data also show that, shoots cultured on semi- solid medium gave the longest formed roots (3.12 and 3.76cm) for sour orange and volkamer lemon rootstocks; while, in sour orange liquid medium (control) was superior than solid one (2.05cm) against (1.37cm). However, in volkamer lemon solid medium gave roots longer than liquid medium (2.96 against 2.37cm).

As for the effect of sampling date on length of formed roots, March date was superior in this concern than December one (2.63 and 3.56 cm against 1.73 and 2.5 cm) in sour orange and volkamer lemon, respectively.

Interaction concerning root length was statistically significant.

Root thickness:

Recorded data in (Tables 3, 4) show that, thickness of sprouted roots formed on cultured multiplicated shoot significantly varied in response to agar application. As such, in both examined rootstocks, solid M.S medium increased thickness of adventitious formed roots than semi-solid or liquid M.S media (0.95, 0.75 and 0.65 mm) for sour orange and (1.00, 0.92 and 0.62mm) for volkamer lemon in descending order. In addition, date of explant excision had a clear effect concerning thickness of sprouted roots. December samples gave thick roots as compared with samples of March (0.81 against 0.75 mm and 0.91 against 0.78 mm) for sour orange and volkamer lemon, respectively. Root thickness increased by increasing agar concentration.

Interaction concerning root thickness as affected by date of sampling and agar application was statistically significant.

Shoot/root ratio:

Ratio between shoot length (cm) and root length (cm) (shoot/

root ratio) was calculated and tabulated in percentages. Shoots cultured in liquid (control) M.S medium (without agar) increased (%) of shoot /root ratio to the maximum (67.75 and 55.30%) followed by shoots cultured on solid M. S medium (55.0 and 48.0%), then the lowest percentage was obtained from shoots cultured on semi –solid M.S medium (42.27 and 33.07%) in descending order. Moreover, shoots of December gave the highest (%) of shoot /root ratio as compared with March ones (58.51 against 53.50 and 52.18 against 38.73%) for March and December in sour orange and volkamer lemon, respectively and actually differed in this concern.

Interaction in this concern was statistically significant.

The obtained herein results proved that, basic M.S medium supplemented with agar only failed to give any roots; while, M.S medium contained proper growth regulators with or without agar gave roots in different tested treatments.

Although, the obtained data in both examined rootstocks concerning rooting (%), number, length and thickness of formed roots and shoot/root ratio were superior in semi-solid medium as compared with liquid or solid media either samples taken in March or December. However, root thickness was increased in cultured shoots on solid medium than those grown in liquid or semi-solid media.

The available literature concerning the effect of agar added to M.S medium is rare. However, many reports (Singh *et al.*, 1994) proved that, multiplicated shoots >1.5 cm of citremon 1452 derived from stem segments with 2 buds from *in vitro* plants were rooted in liquid M.S medium. In the same direction, Harada and Murai. (1996). found that, plant regeneration occurred on solid M.S. medium with 0.8 % agar and Ribeiro *et al.* (1999) showed that, *in vitro* culture of natal embryos were cultured on M.S medium with agar at 0.0, 3.5, 7, 10.5 or 14 g/l and pHs adjusted to 3.7, 4.7, 5.7 or 6.7. The best growth (length of aerial parts and root growth) was observed on media containing agar at 8.7g/l, although the best survival was observed on a medium containing agar at 9.7g/l and adjusted to pH (6.7). The fresh weight of cultured explants decreased by increasing agar concentration.

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الإكثار المعملي (الدقيق) لأصلين من الموالح بأسلوب زراعة القمم النامية
٣- دراسة التجذير

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أجريت التجربة فى معمل زراعة الأنسجة بقسم البساتين - كلية الزراعة - جامعة الزقازيق خلال عامى (٢٠٠١، ٢٠٠٢) وفيها تم اختبار الأفرع المتضاعفة لكل من أصلى النارنج والفولكاماريانا والتى يزيد طولها عن (٥،١سم) لدراسة قدرتها على التجذير، حيث تم زراعتها على بعض بيئات التجذير والتى تتكون أساسا من بيئة موراشيج وسكوج (M.S) (١٩٦٢)، والمزودة ببعض منظمات النمو مثل نفثالين حامض الخليك (NAA) بتركيزات (صفر ، ٠,٥ ، ١ ، ٢ ، ٥ ملجم/ لتر) ، وأندول بيوتريك أسيد (IBA) بتركيزات (صفر ، ٥ ملجم/ لتر) ، وأندول أستيك أسيد (IAA) بتركيزات (صفر ، ٢ ملجم/ لتر)، وذلك بجانب بعض التركيزات المختلطة من هذه المنظمات.

ولقد وجد أن الأفرع المزروعة على بيئة موراشيج وسكوج (M.S) بدون إضافة منظمات النمو فشلت فى إعطاء أى جذور فى كلا الأصلين، وعلى العكس من ذلك، وجد أن إضافة ٥ ملجم/ لتر من NAA (لأصل النارنج) إلى بيئة M.S ، ٥ ملجم/ لتر IBA (لأصل الفولكاماريانا) أعطت أعلى القيم للجذور المتكونة (٢٥,٤٨%)، (٣٢,٥١%) على التوالى. وبالإضافة إلى ذلك، كانت أعلى قيم لعدد الجذور المتكونة/ فرع فى أصل النارنج مع الأفرع المنزرعة على بيئة M.S مضافاً إليها ٥ ملجم/ لتر NAA، والبيئة المضاف إليها IBA بتركيز ٥ ملجم/ لتر بالنسبة لأصل الفولكاماريانا، بينما تراوحت القيم الأخرى بين الكنترول وهذه القيم.

كما أوضحت النتائج أن المنفصلات النباتية (Explants) التى أخذت فى شهر مارس (فترة النشاط) تزداد فيها النسبة المئوية للتجذير، وكذلك عدد الجذور المتكونة/ فرع، وطولها وذلك بالمقارنة بالمنفصلات النباتية المأخوذة فى شهر ديسمبر (فترة السكون)، وكان ذلك واضحاً فى كلا الأصلين تحت ظروف معاملات الدراسة.

وأوضحت النتائج أيضا تأثر سمك الجذور المتكونة بنوع وتركيز منظم النمو المستخدم وكذلك ميعاد أخذ المنفصلات النباتية فى كلا الأصلين المختبرين حيث يزداد سمك الجذور مع المنفصلات النباتية المأخوذة فى ديسمبر عند مقارنتها بميعاد مارس وذلك تحت ظروف المعاملات المختلفة.

كما أوضحت النتائج المتحصل عليها أن إضافة الأجار إلى بيئات التجذير المزودة بمنظمات النمو التى سبق ذكرها يحسن من نمو وتطور الجذور المتكونة على الأفرع، وكانت البيئة شبه الصلبة هى الأكثر ملاءمة لتكوين ونمو وتطور الجذور عند مقارنتها بالبيئة الصلبة أو البيئة السائلة وذلك فى كلا الأصلين المختبرين تحت ظروف معاملات الدراسة المختلفة.