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IN *VITRO* PROPAGATION OF SOME FRUIT SPECIES: A- *IN VITRO* PROPAGATION OF MULBERRY *(Morus alba, L.)* TREES.

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ABSTRACT

A factorial experiment was carried out in tissue culture laboratory, Horticulture Research Institute, Agriculture Research center during the two successive 2002 and 2003 seasons to find out an ideal method of propagation through tissue culture technique. In this concern, shoot tips and nodal segments of 0.8 – 1.0 cm in length were prepared from mature Mulberry trees. After sterilization, the explants were initiated on B_5, MS and WPM media at the strength of full; half and quarter The three media were supplemented with 0.1 mg/L IBA; 1.0 mg/L BA. After four weeks, MS medium gave the best survival percentage and growth parameters, full strength media proved to be the more suitable for the three measurements (survival %, shoot length and number of leaflets). Shoot tips surpassed nodal explants during two seasons of study. On the other hand, nodal cuttings which cultured in quarter WPM medium had the lowest value in this concern. The newly formed shoots were transferred to the same media supplemented with either BAP; Kinetin or 2ip at the concentration of 2, 4 or 6 mg/L through proliferation stage. Full strength MS medium supplemented with 2 mg/L BAP was the superior and had the greatest number of shoots. While the reverse was true with kinetin at 6 mg/L to full strength of B5, MS and WPM media. Micro-shoots were rooted in the same half strength media with or without activated charcoal and supplemented with either IBA at the concentrations of 2, 4, 6 mg/L or NAA at the concentration of 1, 2, 3 mg/L or combination of both at 4 mg/L IBA and 2 mg/L NAA. The plantlet grown on MS medium with activated charcoal supplemented with 6 mg/L IBA gave the highest value of rooting % and number of roots. Meanwhile, adding 4 mg/L IBA to half strength MS medium with activated charcoal proved to be the most effective in increasing the root length. On the other hand, the least value of rooting % were coupled to the charcoal omitted WPM supplemented with 1 mg/L NAA. While, the least number of roots/plantlet was found by charcoal omitted half strength WPM provided with IBA at 2 mg/L. In addition, the half strength MS rooting medium supplemented with IBA 2 mg/ IBA without activated charcoal showed the shortest rootlet. The plantlet produced from the best treatment of each media during the rooting stage were transplanted in (300 ml) plastic pots containing autoclaved vermiculite, peatmoss and sand mixture by volume (1:1:1) as transplanting medium. The plantlets produced from MS medium with activated charcoal supplemented with 6 mg/L IBA recorded the highest survival %, shoot length and number of leaves during two seasons of study. While plantlets cultured in half strength WPM with activated charcoal supplemented with 6 mg/L IBA had the lowest value in this respect the least value of rooting % were coupled to the charcoal omitted WPM supplemented with 1 mg/L NAA. While the least number of roots /plantlet was found by half strength WPM supplemented with IBA at 2 mg/L. In addition, the half strength MS rooting medium supplemented with IBA 2 mg/L without activated charcoal showed the shortest rootlets.

INTRODUCTION

Mulberry (*Morus alba*, L.) is one of the important economic species belonging to the family *Moraceae*. Mulberry is a fast growing deciduous tree grows successfully in sub – tropical, tropical and temperate climates. It is growing digenously in the north and west Asia, Thailand, Malayo, Burma, Bangladesh, India, Pakistan, Turkey, north Iran and Armenia.

No one can deny the economic importance of mulberry; its fruit is eaten, besides the importance in wood industry as well as sericulture industry; the leaves being used to feed silkworms. Sericulture industry is starting to progress in our country and hence, it deserved our interest and study.

Mulberry cultivation is the agricultural part of sericulture industry which constitutes not only the rearing of silkworm but also silk reeling. Cultivation of mulberry plays a significant role in determining the production cost of cocoons and silk as it is estimated that 60 % of the cultivation total cost of cocoons goes to mulberry.

One of the most promising advanced tissue culture technologies is the *in vitro* cloning or asexual propagation of plants. Therefore, an efficient procedure micropropagation of the selected cultivars in a short period of time is required (Sharma and Thorpe, 1990). We propose for this study to provide mulberry transplants to grow on a large scale, especially those are used for sericulture industry as *Morus alba (*mulberry) cultivars.

MATERIALS AND METHODS

The present study was conducted in the Tissue Culture Laboratory, Horticulture Research Institute, Agriculture Res. Center during two seasons of 2002 and 2003. Generally, the following experiments were carried out:

I. Establishment stage:

In this stage, it was aimed to determine the suitable explant type (shoot tip & nodal cutting); kind of media (MS Murashige and Skoog, (1962); B5 Gamborg, *et al.*, (1968) and WPM Lloyd and McCown (1980)) and media strength (full; half and quarter) by which more success could be achieved through the direct regeneration.

New mulberry growing shoots were taken at the beginning of the growing season (early March), washed with running water, and cut into either shoot tips or nodal cutting with about 10 mm length for each. Then explants were washed with tap water for one) at 20 % with two drops of tween-20 for 20 minutes, then in mercuric chloride at hour and soaked in a commercial bleach "Clorox" (5.25 % sodium hypochlorite the concentration of 0.1 % for 10 minutes, and then rinsed three times in sterilized distilled water for ten minutes to remove any residues of Clorox or mercuric chloride.

The sterilized explants were cultured on the three used nutrient media (MS, B_5 or WP) each supplemented with 3 % sucrose; 0.1 mg/L IBA; 1.0 mg/L BA; (6- benzyl adenine) and purified agar (Bacto-Difco agar) at 0.7 %. The pH of the media was adjusted to (5.6 to 5.8). Then, the media dispended

into 100 ml glass jar each contained 25 ml medium then wrapped with plastic screw cap and sterilized. The media were autoclaved at (15 lb/in^2) and 121°C for 20 minutes. All cultures were incubated under conditions of 25°c ± 2 and 16 hours (fluorescent light at 30 µM/ hz /sc) and 8 hours darkness. After 4 weeks, data on survival % of cultured explants; browning; shoot length and No. of leaflets per shoot in response to investigated treatments were recorded.

2- Proliferation "shoot multiplication" stage:

Plant materials needed for this stage were provided from those proliferated shoots newly emerged throughout the previous stage i.e. establishment " 1st stage " Hence, regenerated shoots of both shoot tip and nodal cutting were collected and cultured preliminary on the solid (MS), (B_5) and (WP) media supplemented with several growth regulators i.e., combinations of the cytokinin with auxin, (0.1mg/L) IBA, (30 g/L) sucrose and one of 3 cytokinins i.e., kinetin; BA (6- benzyl adenine) or 2ip (isopentel adenine) at concentration of (2,4,6 mg/L) for each . Each medium (MS, B_5 and WP) was supplemented with (100 mg/L) myo-inositol, 3 % sucrose, pH was adjusted at 0.7 %. Media were autoclaved at (1.5 kg / cm^2) and 121°C for 20 min, then left to cool 24 hrs, before using all cultures were incubated under culture condition.

A factorial experiment using the complete randomized design with three replications was conducted for arranging the 27 investigated treatments i.e, various combinations between 3 media types x3 cytokinin kinds x 3 concentrations of growth regulators (6, 4 and 2mg) treatments. Every replicate was represented by five jars, each contained (40 ml) medium and 2 cultured explants. The number of proliferated shootlets per each original one through three subcultures treatment were recorded.

3- Rooting stage:

Proliferated shoots were taken and separated from each other under aseptic conditions and sub-cultured on half-strength (MS), (B_5) and (WP) media supplemented with (30 g/L) sucrose and (7 g/L) purified Bacto - Difico agar with activated charcoal (1 g/L) or without, media were also varied pertaining the investigated auxin treatments (Kind & level) i.e., IBA at 2, 4 , 6 mg/L; NAA at the 1, 2, 3 mg/L or combination of both 4 mg/L IBA and 2 mg/L NAA. pH was adjusted at (5.6-5.8) and the media were autoclaved and cultures were incubated under culture condition. Elongated shoots were transferred (cultured) in jars containing (40 ml) of the abovementioned rooting media, then incubated for one week in the dark followed by 3 weeks in light.

After four weeks from incubation; rooting %; number of rootlets/ plantlet and average length (cm.) of each were recorded.

4-Acclimatization stage:

Produced Mulberry plantlets were washed with tap water (Ebida, 1991 and Fassuliotis and Nelson, 1992) then dipped in Rhizolix solution (1.0 g/L) as a fungicide for (10 min) just before transplanting in (300ml) plastic pots

containing autoclaved transplanting medium (vermiculite: peat moss: sand) at (1:1:1) and maintained in green house for four weeks.

Pots were arranged then covered with polyethylene bags to maintain high relative humidity percentage around the plants in green house (Fassuliotis and Nelson, 1992). After two weeks, the polyethylene bags were partially removed to allow air circulation (Ali *et al.*, 1990), and later removed after other two weeks from those plantlets (Smith, 1981). Plantlets were irrigated with half strength (MS, B_5 and WP) maintenance medium (free hormone medium) during the period of hardening (Ebida, 1991). The irrigation was applied depending on the requirement of plantlets. Pests and disease control program was controlled as recommendations.

Data were recorded after one month of transplanting as follow:

1- Survival percentage.
2- Plant length (cm).
3- Number of leaves / plant.

Statistical analysis:

Data obtained were statistically analysed according to (Snedecor and Cochran, 1980) and significant differences among means were determined by using Duncan's multiple range test at 5% level of probability(Duncan, 1955).

RESULTS AND DISCUSSION

1-Establishment stage:

1- Browning percentage:

With regard to the data of specific effect of the different factors involved in this study i.e., explant type, media strength and media type on browning percentage, are presented in Table (1). It could be noticed that each factor affected significantly browning %. Herein, the quarter strength media exhibited the highest value of browning, followed in a decreasing order by half strength media then full strength media which showed the least value during the two seasons of the study.

With respect to the specific effect of media type on browning percentage. Results in table (1); Gamborg (B_5) medium showed a significant increase browning percentage followed in a decreasing order by woody plant (WP) medium and MS medium which showed the least value during both seasons. As for the specific effect of explant type, Table (1) showed that browning % was significantly lower with nodal cuttings below shoot tips.

Concerning the interaction effect, data revealed that, browning percentage was significantly responded to interaction effect of various combinations. Whereas, the least value of browning was observed with nodal cutting which cultured on full strength media (MS) medium. The reverse was true with culturing both explant types on the quarter strength B_5 medium. In addition, other combinations were in between the aforementioed two extremes (Table 1).

Table (1): Specific effect of explant type, media strength, media type and interaction effect of their combinations on survival % and browning % of mulberry *Morus alba* during establishment stage (2002 & 2003 seasons).

Explant type	Media strength / Media type	Browning (%)			Mean*	Survival (%)			Mean*
		Full	Half	Quarter		Full	Half	Quarter	
		2002							
Shoot tip	B5	8.50fg	10.20 cd	11.10a	9.53A	70.67b	61.67de	58.67g	64.74A
	MS	8.00h	9.50e	10.00d		75.33a	68.67c	62.33d	
	WP	8.33g	9.67de	10.50c		68.67c	59.33f	57.33g	
Nodal cutting	B5	8.25gh	9.33ef	10.83b	8.90B	70.33b	61.00e	58.00g	64.07B
	MS	7.67i	9.00f	9.25d		75.00a	68.63.c	61.67de	
	WP	7.83hi	8.67fg	9.31ef		67.67c	58.33g	56.33h	
Mean **		8.10C	9.40B	10.17A		71.28A	62.89B	59.06C	
Mean ***		B5	MS	WP		B5	MS	WP	
		9.70A	8.90C	9.05B		63.39B	68.56A	61.28C	
		2003							
Shoot tip	B5	9.30f	10.55de	11.25a	10.11A	71.00b	61.33e	58.83gh	64.83A
	MS	8.66gh	10.25e	10.83c		76.33a	68.33d	62.00e	
	WP	8.83g	10.30e	11.00b		68.33d	60.00f	57.67hi	
Nodal cutting	B5	9.67ef	9.83ef	10.67d	9.41B	69.67c	60.33f	58.33gh	64.15B
	MS	8.15hi	9.20fg	10.25e		75.67a	68.33d	62.33e	
	WP	8.83h	9.28f	10.33de		67.33d	58.67g	56.67i	
Mean **		8.66C	9.90B	10.72A		71.39A	62.78B	59.31C	
Mean ***		B5	MS	WP		B5	MS	WP	
		10.21A	9.56C	9.68B		63.25B	68.78A	61.45C	

*,**,*** Refer to specific effect of explant type, media strength and media type , respectively. Capital and small letter / s were used for distinguishing between values of specific and interaction effects, respectively whereas means followed by the same letter/s were not significantly different at 5 % level.

2-Survival percentage:

Regarding the specific effect of explant type, Table (1) clearly showed that, shoot tips had greater value of survival percentage, than nodal cutting during 2002 & 2003 experimental seasons.

Referring the specific effect of media strength on survival %, The highest significant % was found by full media strength followed in a decreasing order by half media and quarter media strength during the two seasons of study.

As for the specific effect of media type, it is quite evident to be noticed that (MS) medium was the superior i.e, showed the highest value of survival percentage followed in a decreasing order by (B_5) medium while, (WP) medium was the inferior during 2002 and 2003 seasons.

Concerning the interaction effect; data presented in Table (1) and photo (1& 2) displayed obviously that, the both shoot tip and nodal cutting which cultured on full strength MS medium had the highest value. However, the reverse was found with nodal cutting cultured on quarter media strength, especially (WP) medium during 2002 and 2003 seasons. In addition, other combinations were in between the aforementioned two extremes during the study.

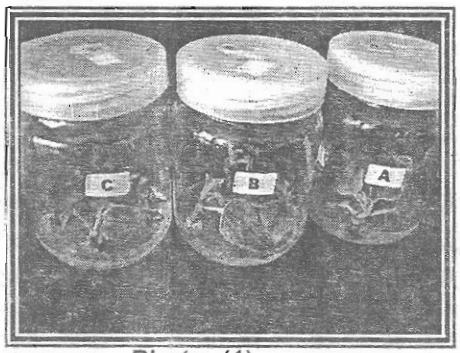

Photo. (1)

Photo. (2)

Photos. (1&2): Effect of explant type, media type and media strength on some measurements during establishment stage of mulberry (*Morus alba, L.*)

1- **Shoot tip in full strength media (A : MS , B: B_5 , C : WP)**
2- **Nodal cutting in full strength media (A : MS, B: B_5, C : WP)**

3-Shoot length (cm.) and number of leaflets/shoot:

Concerning the specific effect of the different factors involved in this study i.e., explant type, media strength and media type on shoot length (cm)

and number of leaflets/shoot Table (2) shows that, the tallest shootlets and number of leaflets were those of shoot tip explants of mulberry (*Morus alba*, L.).While nodal cutting induced shorter shootlets of lower number of leaflets per each during the two seasons of study.

Referring the specific effect of media strength on shoot length and number of leaflets for mulberry, data revealed that, full strength media resulted significantly in the tallest mulberry shoot of higher and number of leaflets followed in a decreasing order by half media strength and quarter media strength during 2002 & 2003 seasons.

As for the specific effect of media type, Murashige and Skoog (MS) medium proved to be the best medium for the growth of mulberry, followed in a decreasing order by Gamborg (B_5) medium which ranked second, however woody plant (WP) medium significantly exhibited the shortest shoot length and lowest number of leaflets during 2002 & 2003 seasons.

Concerning the interaction effect; data obtained as shown from Table (2) and photo (1&2) displayed obviously that, shoot tip of mulberry (*Morus alba*), cultured on full strength media (MS) medium exhibited statistically the tallest shoot and highest number or leaflet/shoot. The reverse was true with the nodal cutting on quarter strength of (WP) medium during 2002 and 2003 experimental seasons. In addition other combinations were in between the above mentioned two extremes. It is easy to say that, during establishment stage results of this investigation indicated that culturing shoot tips on full strength (MS) medium enhanced survival %, shoot length, number of leaflets per shoot. In the same time stem nodal cutting was significantly more resistant to browning on full strength of (MS) medium. These results were in agreement with those reported by Kim *et al.*, (1985), Menard *et al.*, (1985); Ivanicka (1987) and Zaman *et al.*, (1998), on mulberry Saker *et al.*, (1999); Schuch *et al.*, (2003) and Soliman (2004) on different fruit trees.

II-Multiplication stage:

In this concern, specific effect of three studied factors i.e., media type (MS, B_5 and WP); concentration of growth regulators (2, 4 and 6mg/L); growth regulators type (BA, 2ip and Kinetin) and their possible combinations were investigated pertaining the response on number of proliferated shoots through 3 subcultures of multiplication stage data obtained are Presented in Table (3).

Concerning the specific effect of media type, it is quite clear as shown from Table (3) that Murashige & Skoog (MS) medium was the superior through three subcultures where the greatest number of shoots was induced followed statistically by Gamborg (B_5) medium, while woody plant (WP) medium was the inferior, during two seasons of study.

Regarding the specific effect of growth regulator concentrations, added to multiplication media Table (3) shows that the least concentration (2mg /L) resulted significanlty in the highest values of number of shoots, followed in a decreasing order by the intermediate concentration of growth regulators at (4mg/L), while the reverse was detected with (6mg /L) during 2002 & 2003 seasons.

Table (2): Specific and interaction effects of explant type, media strength, media type and their combinaions on shoot length (cm.) and No. of leaflets/shoot of mulberry *Morus alba* during establishment stage (2002 & 2003 seasons).

Explant type	Media strength / Media type	Shoot length (cm.) Full	Half	Quarter	Mean*	No. of leaflets/shoot Full	Half	Quarter	Mean*
2002									
Shoot tip	B5	2.01b	1.72c-e	1.36ij		3.25bc	2.83d	2.25e-g	
	MS	2.34a	1.76cd	1.48gh	**1.68A**	3.75a	3.08cd	2.5e	**2.61A**
	WP	1.73c-e	1.51gh	1.18k		2.20e-g	2.15g	1.50h	
Nodal cutting	B5	1.82c	1.59fg	1.30j		3.00cd	2.42ef	2.16fg	
	MS	1.97b	1.67d-f	1.43hi	**1.54B**	3.50ab	2.80d	2.40ef	**2.43B**
	WP	1.63ef	1.41h-j	1.05l		2.18e-g	2.10g	1.33h	
Mean **		**1.92A**	**1.61B**	**1.30C**		**2.98A**	**2.56B**	**2.02C**	
Mean ***		**B5**	**MS**	**WP**		**B5**	**MS**	**WP**	
		1.63B	**1.78A**	**1.42C**		**2.65B**	**3.01A**	**1.91C**	
2003									
Shoot tip	B5	1.90b	1.40fg	1.25i		3.17c	2.75d	2.17f-h	
	MS	2.35a	1.76c	1.49f	**1.61A**	3.50b	3.08c	2.33f	**2.50A**
	WP	1.72cd	1.45f	1.15j		2.16f-h	2.00h	1.35j	
Nodal cutting	B5	1.80c	1.61e	1.28hi		3.16c	2.15f	2.08gh	
	MS	1.93b	1.63de	1.35gh	**1.53B**	3.67a	2.38e	2.30f	**2.40B**
	WP	1.61e	1.44fg	1.08j		2.18fg	2.13h	1.58i	
Mean **		**1.89A**	**1.55B**	**1.27C**		**2.99A**	**2.42B**	**1.97C**	
Mean ***		**B5**	**MS**	**WP**		**B5**	**MS**	**WP**	
		1.54B	**1.75A**	**1.41C**		**2.59B**	**2.88A**	**1.90C**	

*, **, *** Refer to specific effect of explant type, media strength and media type , respectively. Capital and small letter / s were used for distinguishing between values of specific and interaction effects, respectively whereas means followed by the same letter/s were not significantly different at 5 % level.

Table (3) : Specific effect of media type, concentration of growth regulators, kind of growth regulators and interactioneffect of their combinations on number of shoots (4 weeks later) during multiplication stage of jojoba (*Simmondsia shinensis)* (2002 & 2003 seasons).-

Media type	Number of shoots / Growth regulators type	Frist Sub. Concentrations of growth regulators 2	4	6	Means*	Secound Sub. Concentrations of growth regulators 2	4	6	Means*	Thrid Sub. Concentrations of growth regulators 2	4	6	Means*
2002													
B5	**BA**	4.67 d	3.66g	2.16jk	**2.92B**	6.30c	4.67f	2.29kl	**3.30B**	7.00c	4.67f	2.90j	**3.70B**
	2iP	4.17ef	3.65g	2.00k		4.66f	3.33h	2.16lm		4.64f	4.00g	2.58l	
	Kinetin	2.48i	2.10k	1.42mn		2.70i	2.10m	1.50n		3.16i	2.66kl	1.67p	
MS	**BA**	7.66a	5.67c	2.50i	**3.93A**	7.67a	5.67d	2.75i	**4.09A**	7.67a	5.67d	3.33i	**4.44A**
	2iP	6.67b	4.33e	2.25j		6.67b	5.00e	2.50jk		7.33b	5.00e	2.92j	
	Kinetin	2.58i	2.17kl	1.58lm		2.60ij	2.31kl	1.67n		3.30i	2.83jk	1.83op	
WP	**BA**	4.00f	3.00h	1.98k	**2.48C**	5.45d	3.30h	2.09m	**2.95C**	5.60d	3.67h	2.33m	**3.29C**
	2iP	3.60g	2.60i	1.72l		4.33g	3.28h	2.00m		5.00e	3.33i	2.17mn	
	Kinetin	2.45i	1.69l	1.25n		2.55ij	2.08m	1.48n		2.87j	2.75j-l	1.95no	
Mean**		**4.25A**	**3.21B**	**1.87C**		**4.77A**	**3.53B**	**2.05C**		**5.17A**	**3.85B**	**2.41C**	
Mean***		**BA**	**2iP**	**Kinetin**		**BA**	**2iP**	**Kinetin**		**BA**	**2iP**	**Kinetin**	
		3.92A	**3.44B**	**1.98C**		**4.46A**	**3.77B**	**2.11C**		**4.76A**	**4.11B**	**2.56C**	
2003													
B5	**BA**	5.00c	3.55e	2.33f-h	**3.00B**	6.00bc	4.00hi	2.32k-m	**3.241B**	6.67c	4.66f	3.00jk	**3.66B**
	2iP	4.31d	3.50e	2.15g-l		4.60fg	3.67i	2.15l-n		4.60f	3.67gh	2.67k-m	
	Kinetin	2.66fg	2.18g-l	1.33j		2.83jk	2.17l-n	1.42o		3.29h-j	2.66k-m	1.75o	
MS	**BA**	7.67a	5.17c	2.42fg	**3.88A**	7.66a	5.66cd	2.67jk	**4.037A**	8.33a	5.83d	3.17i-k	**4.62A**
	2iP	6.66b	4.50d	2.20g-i		6.33b	5.10ef	2.33k-m		7.66b	5.33e	2.91j-l	
	Kinetin	2.67fg	2.10g-l	1.58j		2.66j-l	2.30k-m	1.66no		3.50g-l	2.80j-m	2.00no	
WP	**BA**	4.30d	3.67e	1.83h-j	**2.600C**	5.33de	3.67i	1.92m-o	**2.972C**	5.50de	3.83g	2.42l-n	**3.313C**
	2iP	3.62e	2.75f	1.71ij		4.30gh	3.00j	2.12l-n		4.55f	3.50g-l	2.30mn	
	Kinetin	2.58fh	1.67ij	1.33j		2.65j-l	2.25k-n	1.42o		2.88j-l	2.70k-m	2.08no	
Mean**		**4.39A**	**3.23B**	**1.87C**		**4.71A**	**3.53B**	**2.00C**		**5.22A**	**3.90B**	**2.48C**	
Mean***		**BA**	**2iP**	**Kinetin**		**BA**	**2iP**	**Kinetin**		**BA**	**2iP**	**Kinetin**	
		3.99A	**3.49B**	**2.01C**		**4.36A**	**3.73B**	**2.15C**		**4.82A**	**4.13B**	**2.63C**	

*,**,*** Refer to specific effect of media type, concentration of growth regulators and kind of growth regulators, respectively.Capital letter / s and small letter/s were used for distinguishing between values of specific and interaction effects, respectively whereas means followed by the same letter/s were not significantly different at 5 % level.

With respect to the specific effect of growth regulators kind, it is also clear that, the BA supplemented medium, had the greatest values of proliferated shoots during the 2002 & 2003 seasons. On the contrary, kinetin was the inferior as it induced significantly the least number of shoots.

Concerning the Interaction effect, Table (3) and photo (3) display that the greatest number of proliferated shoots was always in concomitant to the Murashige & Skoog (MS) medium supplemented with (BA) at the concentration of 2 mg /L during 2002 & 2003 seasons.

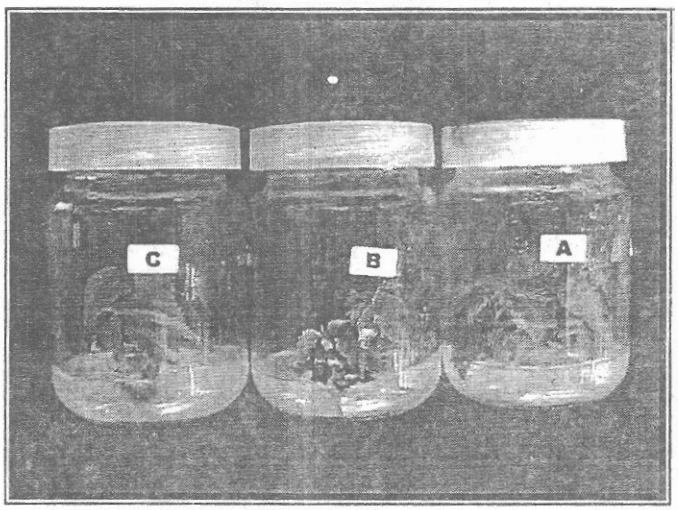

Photo. (3)

Photo. (3): Effect of growth regulators added to three media through the 3rd subculture within multiplication stage of mulberry (*Morus alba, L.*)

A: Cultured explant in MS medium supplemented with (2mg /L) BA
B: Cultured explant in B_5 medium supplemented with (2mg /L) BA
C: Cultured explant in WP medium supplemented with (2mg /L) BA

The reverse was found with combinations of culturing on (WP) medium supplemented with 6 mg /L Kinetin. In addition other combinations were in between the aforesaid two extremes during the three subculture in the two seasons of study. These results go in line with those found by Chang (1985); Zaman *et al.*, (1998), on mulberry; Sun *et al* (2000); Erig and Schugh (2003) and Soliman (2004) on some fruit species.

III- Rooting stage:

In with respect adding two auxins (IBA or NAA) each either solely at three levels (2, 4, and 6 mg/L for IBA and 1, 2, and 3 for NAA, or combined together (IBA at 4 mg/L + NAA at 2 mg/L) to one half strength of the three B_5, MS and WP media supplemented with 1.0 gm/L activated charcoal or not in combination were investigated after 4 weeks from incubation through rooting stage regarding the influence on rooting percentage, number of root per plantlet and average root length of mulberry (*Morus nigra*), Data obtained are presented in Tables (4 & 5) and illustrated by Photo. (4).

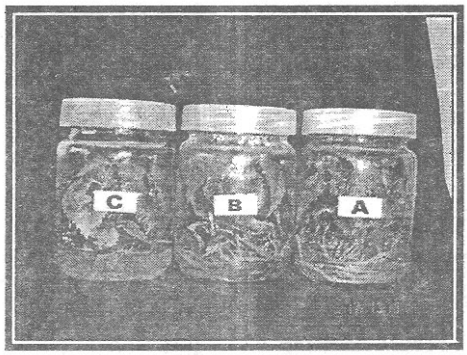

Photo. (4)

Photo. (4): Effect of media type and auxin treatments on some measurements during rooting stage of mulberry (*Morus alba, L.*)

A: Cultured shootlets in 1/ 2 strength MS + IBA (6mg/L) without activated charcoal

B: Cultured shootlets in 1/ 2 strength B_5 + IBA (6mg/L) without activated charcoal

C: Cultured shootlets in 1/ 2 strength WP + IBA (6mg/L) without activated charcoal

1- Rooting percentage:

Referring the specific effect of media type on the rooting percentage, data revealed that, Murashige and Skoog (MS) medium gave the highest value of rooting percentage, followed in a decreasing order by Gamborg (B_5) medium, while Woody plant (WP) medium was the inferior during 2002 & 2003 seasons.

Regarding the specific effect of activated charcoal added to half strength media, Table (4) shows that, the presence of activated charcoal exhibited higher rooting % during 1st and 2nd seasons.

Concerning the specific effect of auxin treatments (kind and rate) added to media, obtained data displayed that adding IBA at (6 mg/L) was the superior, statistically followed in a decreasing by adding NAA at (3 mg/L) and IBA at 4 mg/L which ranked second and third, respectively. While adding IBA at 2 mg/L was the inferior and ranked last during 2002 and 2003 seasons. Differences between these 7 treatments were significant when comparing each other during the study.

From the obtained results, it was so worthy to be noticed that the effect of adding auxin (kind and rate) to half strength-rooting medium varied not only from one supplemented auxin to another, but also depended upon the concentration of added auxin itself. Accordingly, the highest levels (6 mg/L) IBA and (3mg/L) NAA) were more effective. While the reverse was true with the lowest level IBA at (2mg/L), NAA at (1mg/L), while mixture of IBA at (4mg/L) + NAA at (2mg/L) was intermediate during the study .

Table (4): Specific and interaction effects of media type; activated charcoal; auxin treatments (kind & concentration) added to one half strength rooting medium and their combinations on rooting percentage during rooting stage of mulberry (Morus alba, L.) after 4 weeks incubation during (2002 & 2003 seasons).

rooting percentage									
Media type	Auxin Treatments	A.Ch.		Mean *	Mean **	A.Ch.		Mean *	Mean **
		With	Without			With	Without		
		2002				2003			
B5	IBA 2mg/L	71.00u	68.67w	76.09B	IBA 2mg/L 69.63E	70.95r	68.60u	76.05B	IBA 2mg/L 69.46F
	IBA 4mg/L	77.30k	75.33m			77.56hi	75.67j		
	IBA 6mg/L	86.33b	83.60d			86.33b	83.67d		
	NAA 1mg/L	69.33v	66.67x		IBA 4mg/L 75.98C	69.30t	66.60v		IBA 4mg/L 76.08C
	NAA 2mg/L	76.33l	73.80op			76.00j	73.33mn		
	NAA 3mg/L	83.30de	81.33g			83.29d	81.67f		
	IBA 4mg/L + NAA 2mg/L	77.59jk	74.67n		IBA 6mg/L 83.14A	77.67hi	74.00l		IBA 6mg/L 84.32A
MS	IBA 2mg/L	73.00qr	71.10u	77.42A		72.99no	70.33s	77.21A	
	IBA 4mg/L	80.67h	78.00ij			80.33g	77.67hi		
	IBA 6mg/L	88.00a	85.30c		NAA 1mg/L 68.05F	87.67a	85.00c		NAA 1mg/L 67.92G
	NAA 1mg/L	71.67st	69.60v			71.33qr	69.67t		
	NAA 2mg/L	77.60jk	75.33m			77.33i	75.67j		
	NAA 3mg/L	85.00c	83.00e		NAA2mg/L 74.11D	85.33c	83.33d		NAA2mg/L 73.96D
	IBA 4mg/L + NAA 2mg/L	74.00o	71.60st			72.95no	71.30qr		
WP	IBA 2mg/L	68.33w	65.67y	72.88C		68.58u	65.33w	72.89C	
	IBA 4mg/L	73.30pq	71.29yu		NAA3mg/L 81.94B	73.67lm	71.59pq		NAA3mg/L 82.05B
	IBA 6mg/L	82.33f	80.58h			82.67e	80.67g		
	NAA 1mg/L	66.33x	64.67z			66.30v	64.33x		
	NAA 2mg/L	72.00s	69.58v		IBA 4mg/L + NAA 2mg/L 74.19D	72.00p	69.4t		IBA 4mg/L + NAA 2mg/L 73.85E
	NAA 3mg/L	80.67h	78.33i			80.67g	78.00h		
	IBA 4mg/L + NAA 2mg/L	74.58n	72.67r			74.60k	72.67o		
Mean ***		76.60A	74.32B			76.55A	74.21B		

*,**,*** Refer to specific effect of media type, auxin treatments (kind & concentration) and added activated charcoal to one half strength rooting strength medium. Capital and small letter /s were used for between values of speceific and interaction effects, respectively whereas means followed by the same letter/s were not significantly different at 5 % level.

Concerning the interaction effect, Table (4) displays that, rooting % of mulberry (Morus alba, L.) reacted obviously to various (media type x activated charcoal x auxin kinds and concentration). Half strength (MS) rooting medium supplemented with activated charcoal and IBA at (6 mg/L) gained statistically the highest rooting %. On the contrary, the lowest value of rooting % was always in concomitant to the charcoal omitted half strength (WP) rooting medium supplemented with NAA at (1mg/L) during 1st and 2nd seasons. In addition, other combinations were in between the abovementioned two extremes.

These results are in general agreement with the findings previously found by Chen *et al.*, (1998); Magyar *et al.*, (2002); and Soliman (2004) regarding the response to auxin kind and concentration.

However, the present data regarding the effect of presence of activated charcoal in the rooting medium supported in general with the findings of Bondok *et al.*, (1989); Fouad *et al.*, (1995); Magyar *et al.*, (2001) and Soliman (2004).

2-Average root length (cm.):

Concerning the specific effect of different factors involved in this study i.e., rooting media type, presence of activated charcoal and different auxin treatments (kind & concentration) on the average root length (cm.) of mulberry (Morus alba, L.) plantlet. Data presented in Table (5) displayed that, (MS) rooting medium exhibited the tallest roots followed in a decreasing order by (B_5) medium while (WP) medium was the inferior during 1st and 2nd seasons. Differences were significant when average root length of a given media was compared to those of the two other ones. With respect to the specific effect of adding activated charcoal to half strength rooting medium, Table (5) displays, the beneficial effect of adding activated charcoal to the medium whereas root length was obviously increased as compared to the analogous one without charcoal during 2002 and 2003 seasons.

As for the specific effect of auxin (kind and rate) added to either charcoal omitted or supplemented rooting medium on average root length, obtained data in Table (5) displayed that adding (4mg/L) IBA to half strength rooting medium was the superior and exhibited the tallest root in 1st & 2nd seasons. Whereas, adding either NAA at (3mg/L) or IBA at (4mg/L) + NAA at (2mg/L) to half strength rooting media both ranked statistically second in both seasons. On the contrary, IBA at (2mg/L) supplemented to media was the inferior.

In addition other auxins treatments were in between the aforesaid two extents (superior & inferior ones) regarding average root length.

Concerning the interaction effect, Table (5) and Photo (4) revealed that half strength (MS) rooting medium supplemented with activated charcoal plus IBA at (4mg/L) or NAA at (3mg/L) as well as IBA at (4mg/L) + NAA at (2mg/L) treatments showed the tallest root of mulberry (*Morus alba,* L.) plantlets during two seasons of study. On the contrary, adding IBA at the lowest rate (2mg/L) to the activated charcoal omitted half strength (WP) rooting medium gave the shortest roots in 1st and 2nd seasons. In addition, other investigated combinations, were in between as compared to the aforesaid two extremes.

Table (5): Specific and interaction effects of media type; activated charcoal; auxin treatments (kind of concentration) media and their combinations on average root length (cm) and num ber of roots during rooting stage of mulberry *Morus alba* after 4 weeks incubation during 2002 & 2003 seasons.

Media type	Parameters / Treatments	Root length (cm.) A.Ch. With	A.Ch. Without	Mean *
2002				
B5	IBA 2mg/L	5.33r	4.49t	7.64B
	IBA 4mg/L	10.58bc	9.00f	
	IBA 6mg/L	7.92i	6.41p	
	NAA 1mg/L	7.83ij	7.16no	
	NAA 2mg/L	6.42p	4.57t	
	NAA 3mg/L	10.42c	8.30h	
	IBA 4mg/L + NAA 2mg/L	10.75b	7.75i-k	
MS	IBA 2mg/L	5.50r	4.58t	8.11A
	IBA 4mg/L	11.18a	9.50e	
	IBA 6mg/L	8.75g	7.08no	
	NAA 1mg/L	8.25h	7.50k-m	
	NAA 2mg/L	6.92o	5.08s	
	NAA 3mg/L	11.16a	8.42h	
	IBA 4mg/L + NAA 2mg/L	11.25a	8.40h	
WP	IBA 2mg/L	4.56t	3.83v	6.96C
	IBA 4mg/L	9.92d	7.70i-k	
	IBA 6mg/L	7.30l-n	6.10q	
	NAA 1mg/L	6.67p	6.62p	
	NAA 2mg/L	6.08q	4.24u	
	NAA 3mg/L	9.58e	7.58j-l	
	IBA 4mg/L + NAA 2mg/L	10.00d	7.36mn	
Mean ***		8.40A	6.75B	
2003				
B5	IBA 2mg/L	4.75vw	4.42xy	7.66B
	IBA 4mg/L	10.67d	9.17h	
	IBA 6mg/L	8.08kl	6.33s	
	NAA 1mg/L	7.91lm	7.25no	
	NAA 2mg/L	6.50s	4.50wx	
	NAA 3mg/L	10.50d	8.42ij	
	IBA 4mg/L + NAA 2mg/L	10.92c	7.83m	
MS	IBA 2mg/L	5.49u	4.58v-x	8.06A
	IBA 4mg/L	11.170ab	9.55g	
	IBA 6mg/L	8.58i	7.08op	
	NAA 1mg/L	8.25jk	7.42n	
	NAA 2mg/L	6.83qr	4.83v	
	NAA 3mg/L	11.00bc	8.42ij	
	IBA 4mg/L + NAA 2mg/L	11.33a	8.33ij	
WP	IBA 2mg/L	4.74vw	3.83z	6.99C
	IBA 4mg/L	9.67fg	7.67m	
	IBA 6mg/L	7.33n	5.67u	
	NAA 1mg/L	7.00pq	6.75r	
	NAA 2mg/L	6.00t	4.25y	
	NAA 3mg/L	9.75f	7.69m	
	IBA 4mg/L + NAA 2mg/L	10.08e	7.42n	
Mean ***		8.41A	6.73B	

*,**,*** Refer to specific effect of supplemented media type, auxin concentration and added activated charcoal to one distinguishing between values of specific andhalf strength medium and incubation condition respectively. Capital and smail letter /s were used for interaction effects, respectively whereas means followed by the same letter/s were not significantly different at 5 % level.

Generally, it could be concluded that adding activated charcoal to half strength (MS) rooting media supplied with IBA at (4mg/L) or NAA at the rate of (2mg/L) or IBA at (4mg/L) + NAA at (2mg/L), were the most preferable treatments which could be recommended for being applied from the economic standpoint. These results are in general agreement with the findings previously reported by Snir (1984), Vaser *et al.*, (2000), Magyar *et al.*, (2001), Thomas TD (2003).

3- Number of roots per plantlet:

Concerning the specific effect of the different factors involved in this study i.e., media type, activated charcoal, auxin (kind and rate) on number of roots per plantlets, data obtained in Table (5) showed that, half strength (MS) rooting medium gained statistically the highest number of roots followed in a decreasing order by half strength (B_5) rooting medium. On the contrary, the lowest number of roots per plantlet was always in concomitant to half strength WPM rooting medium during 1^{st} and 2^{nd} seasons.

As for the influence of adding activated charcoal to half strength rooting medium, Table (5) displays that the number of roots per plantlet was markedly increased by adding activated charcoal to rooting media as compared to charcoal omitted media during 2002 and 2003 seasons.

Regarding the specific effect of adding various IBA & NAA treatments to half strength rooting medium, it is quite clear as show from Table (5) that the largest number of rootlets per plantlet was markedly related to the half strength medium supplemented with (6 mg/L) i.e., IBA. On the contrary, the lowest number of roots/plantlet was markedly in closed relationship to the half strength medium supplemented with 3mg/l NAA during 2002 and 2003 seasons. In addition, other auxin treatments were in between the aforesaid two extremes.

Concerning the interaction effect, it could be safely concluded that half strength (MS) rooting medium supplemented with activated charcoal and IBA at 6 mg/L gained statistically the largest number of roots per plantlet. On the contrary, the lowest number of roots per plantlet was always in concomitant to the charcoal omitted half strength (WP) rooting medium supplemented with IBA at (2 mg/L) or NAA at 3mg/L during 2002 and 2003 seasons. In addition, other combinations were in between the abovementioned two extremes. These results are a general agreement with the findings of Vasar *et al.*, (2000); Magyar –Tabori *et al.*, (2001); Magyar – Tabori *et al.*, (2002); Rogaliski *et al.*, (2003) *and* Soliman, (2004).

IV- Acclimatization stage:

Acclimization stage: In this stage the plantlets produced by the best treatments through preceeding stage (rooting) were cultivated on acclimization medium consisting of vermiculate, peatmoss and sand at equal proportions by volume. Table (6) and photo (5) display that the highest survival% and vegetative growth value were recorded by such newly developed plantlets rooted on half strength MS medium provided with 6mg/L IBA + 1.0 mg/L activated charcoal, followed in descending order by those of half strength B5 medium supplemented with 6 mg/L IBA + 1.0 mg/L activated

charcoal. However, half strength WP medium supplemented with 6 mg/L IBA+1.0 mg/L activated charcoal took the other away around in this concern. These results are in general agreement with the findings of El-Kazzaz *et al.*, (1997); Hoffmann *et al.*, (1999); Benzioni et *al.*, (2003) and Soliman (2004).

Table (6): Comparison between the most effective three rooting treatments on survival %;shoot length (cm) and number of leaves of newly induced *Morus alba* plantlets acclimatization during 2002 and 2003 seasons.

Parameters	Survival		Shoot length		No. leaves	
Treatments	2002	2003	2002	2003	2002	2003
½ strength MS +IBA(6mg/L) A. C.1g/L	77.60 a	77.67 a	16.67 a	16.68 a	9.67 a	9.33 a
½ strength B5+ IBA(6mg/ L) A. C.1g/L	75.33 b	75.67 b	15.67 b	14.98 b	8.66 b	8.27 b
½ strength WPM+ IBA (6mg/L) A. C.1g/L	74.33 c	74.67 c	14.67 c	14.64 c	7.60 c	7.33 c

Photo. (5)

Photo. (5): Effect of some rooting media types and auxin treatments through rooting stage on acclimatized mulberry (*Morus alba, L.*) plants.

A: Rooted plantlets on 1/ 2 strength MS + IBA (6mg/L) + activated charcoal (1.0g/L)

B: Rooted plantlets on 1/ 2 strength B_5 + IBA (6mg/L) + activated charcoal (1.0g/L)

C: Rooted plantlets on 1/ 2 strength WP + IBA (6mg/L) + activated charcoal (1.0g/L)

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الإكثار بتقنية زراعة الأنسجة لبعض أنواع الفاكهة:

أ- إكثار التوت العماني بتقنية زراعة الأنسجة

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أجريت هذه الدراسة في موسمي ٢٠٠٢، ٢٠٠٣ بمعمل زراعة الأنسجة بمعهد بحوث البساتين على التوت العماني صعب التجذير حيث أجريت هذه الدراسة بهدف محاولة إيجاد طريقة قياسية ومناسبة وسريعة لإنتاج نباتات متماثلة من التوت العماني وعلى ذلك استخدام تكنيك زراعة الأنسجة متضمنا المراحل التالية (الأساس ـــ التضاعف ـ التجذير والأقلمة) كالآتى : ـ

أولا : مرحلة الأساس .

وفي هذا الصدد أجريت تجربة عاملية لدراسة التأثير النوعى لكل من نوع المنفصل النباتى (البرعم الطرفى والعقلة ذات البرعم الواحد) وكذلك نوع البيئة (WPM, MS, B_5) وتركيز أملاحها الأساسية (كاملة، نصف وربع تركيز) على نسبة البقاء ومتوسط طول الفريخات وعدد الوريقات المتكونة فبعد أجراء التعقيم للمنفصلات النباتية تم زراعتها علي البيئات السابقة الذكر مضاف إلى كل منها ٠,١ ملجم / لتر اندول حمض البيوتريك , ١ مجم / لتر بنزيل أدنين . فقد أظهرت النتائج المتحصل عليها الآتي: ـ

- تفوق البرعم الطرفى على العقلة ذات البرعم الواحد في تسجيل أعلى نسبة بقاء وكذلك طول الفريخات وعدد الأوراق المتكون عليها وكانت أفضل البيئات هى بيئة موراشيج وسكوج كاملة القوة وكان العكس صحيحا مع العقلة ذات البرعم الواحد التى تم زراعتها على بيئة الأشجار الخشبية ذات الربع تركيز وذلك للقياسات الثلاثة المختبرة.

ثانيا: مرحلة التضاعف.

فى هذه المرحلة تم إعادة الزراعة ثلاث مرات متتالية وإستمرت كل مرة ٤ أسابيع أستخدمت منفصلات نباتية بعمر ٤ أسابيع ناتجة من المرحلة الأولى (الأساس) حيث تم زراعتها على الثلاث بيئات السابقة بتركيز قوة كاملة المضاف إليها ثلاث أنواع من السيتوكينينات (بنزيل أدنين وأيزوبنتيل أدينين وكينتين) كل بثلاث تركيزات هى ٢-و٤و٦ ملجم / لتر في تباديل وتراكيب مختلفة بينها لدراسة تأثيرها على عدد الأفرع المتكونة وقد أوضحت الدراسة النتائج التالية : ـ

- تفوقت بيئة موراشيج وسكوج فى زيادة عدد التفريعات على بيئة جامبورج وبيئة الأشجار الخشبية.
- البنزيل أدنين بتركيز ٢ ملجم / لتر كان أكثر تفوقا فى هذا الشأن .
- أضافة الكينتين بتركيز ٦ ملجم / لتر على بيئة جامبورج أو الأشجار الخشبية أعطى أقل عدد تفريعات .

ثالثا : مرحلة التجذير .

تم تجذير الأفرع الجديدة والمتكونة في مرحلة التضاعف على البيئات الثلاثة السابقة بوضع تركيز املاحها الأساسية سواء تحتوى على الفحم النشط بتركيز ١ ملجم / لتر أو الخالية منه والمضاف إليها اندول حامض البيوتيريك بتركيز٢و٤و٦ ملجم / لتر أو نفثالين حامض الخليك بتركيز ١و٢و٣ مجم / لتر أو خليط من اندول حامض البيوتيريك (IBA) + نفثالين حامض الخليك بتركيز ٢-٤ ملجم على التوالى حيث قيمت هذة المعاملات بمدى استجابة القياسات الثلاثة (النسبة المئوية للتجذير ـ عدد الجذور لكل نبات ومتوسط طول الجذر) وقد تفوقت بيئة موراشيج وسكوج على بيئتىB5 WPM للقياسات الثلاثة

- إضافة الفحم المنشط إلى البيئة أدى إلى زيادة معنوية للقياسات الثلاثة.
- إندول حامض البيوتيريك بتركيز ٦ ملجم / لتر سجل أعلى القيم للقياسات الثلاثة السابقة الذكر خاصة نسبة التجذير.
- تفوقت بصفة عامة التراكيب بين بيئة MS واضافة الفحم مع IBA او NAA بالتركيز الاعلى بينما كان العكس صحيحا مع بيئة WPM بدون فحم والمزودة ب NAA ١جرام/لتر

رابعا: مرحلة الأقلمة .

في هذه المرحلة أجريت تحت ظروف الصوبة الزجاجية حيث تم نقل نباتات التوت العماني الناتجة من ثلاث معاملات اثناء مرحلة التجذير على البيئات الثلاثة في أصص بلاستيك (٣٠٠ مم) مملوءة بمخلوط معقم من البيت موس والفيرميكوليت والرمل بنسبة حجميه (١:١:١) لمدة ٤ أسابيع لدراسة نسبة البقاء وطول النبات وعدد الأوراق لكل منها وقد أوضحت النتائج المتحصل عليها : ـ

- النباتات الناتجة من مرحلة التجذير على بيئة (MS) المضاف إليها 2 ملجم / لتر إندول حامض البيوتيريك + ١ جم فحم نشط سجلت أعلى نسبة بقاء - طول النبات وعدد الأوراق) وكان العكس صحيحا للنباتات الناتجة من مرحلة التجذير على بيئة WPM والمضاف إليها ٦ ملجم البيوتيريك IBA ( ) لكل من القياسات الثلاثة السابقة .