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IN *VITRO* PROPAGATION OF SOME FRUIT SPECIES.
B- *In vitro* propagation of jojoba (*Simmondsia chinensis* Link, Schnider) plant.

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ABSTRACT

Vegetative propagation of jojoba plant is difficult by traditional methods. A factorial experiment was conducted to develop a protocol for cloning jojoba through tissue culture technique .In this concern, shoot tips and nodal cuttings were prepared from jojoba plants. After sterilization, the explant were initiated on three culture media i.e. MS , B_5 and WPM each at either full , one half or one fourth strength, these media supplemented with 0.1 mg/L IBA, 1.0 mg/L BA for establishment stage. After four weeks, MS medium gave the best results with the three measurements (survival %, shoot length and number of leaflets) full strength media proved to be the more suitable for the three measurements. Shoot tips surpassed nodal cutting explant. On the other hand, nodal cuttings which cultivated in quarter WPM had the lowest value in this respect. The newly formed shoots were transferred to the same media supplemented with either BA; Kinetin or 2ip at the concentration of 2, 4, 6 mg/L for each through proliferation stage. Full strength MS medium supplemented with 2 mg/L BA was superior and had the greatest number of shoots during the three subcultures. While the reverse was true with kinetin at 6 mg/L added to full strength WPM. Micro-shoots were rooted in the same half strength media with or without activated charcoal supplemented with 7 mg/L IBA + 1 mg/L NAA plus either 1 or 1.5 mg/L caffeic acid. The data revealed that WPM was most suitable for the three rooting growth measurements (rooting percentage, number of roots/plantlet and average root length) the presence of activated charcoal increased significantly the three rooting growth measurements IBA at (7 mg/L) + NAA at (1mg/L) + caffeic acid at (1 mg/L) gave the highest value of rooting measurements . While, the reverse was detected by the charcoal omitted B_5 medium supplemented with IBA at (7 mg/L) + NAA at (1 mg/L) + caffeic acid at (1.5 mg/L) during the two seasons of study. The plantlet produced from the best treatments of each medium, during the rooting stage were transplanted to (300 ml) plastic pots containing autoclaved transplanting media (vermiculite : peat moss : sand mixed by volume (1:1:1) and maintained in green house for four weeks to investigate their effect on survival % , plant height and number of leaves per plant during acclimatization stage .The obtained results could be summarized as follows :- rooted plantlet in ½ strength WPM + IBA (7 mg/L) + (1.0 mg/L) NAA + (1 mg/L) caffeic acid + 1.0 mg/L activated charcoal gave the highest value of rooting growth measurements while the reverse was true with rooted plantlet in ½ strength B_5 + IBA (7.0 mg/L) + (1.0 mg/L) NAA + (1.0 mg/L) caffeic acid + 1.0 mg/L activated charcoal.

INTRODUCTION

Jojoba plant (*Simmondsia chinensis*, Link, Schneider) which pronounced as ho-ho-ba belongs to family Simmondisceae. This plant is native to the arid zones of USA and Mexico.

Its natural distribution lies between 25 and 34 latitudes in an area, which closely approximates the Sonorant Desert (Gentry, 1958). Jojoba plant has currently received a special attention since its seeds contain liquid waxy

called jojoba oil. This oil is very similar to that obtained from sperm whale. The liquid wax of jojoba is used as a natural base for wide range of cosmetics and medicinal products, in addition, it has heat resistant lubricating properties and useful in chemical industry (Naqvi *et al.,* 1988).

Clonal propagation exhibited elite individuals of known sexuality and, special relevance in order to make sure of the number of productive plants in a given plot. Its vegetative propagation is difficult by traditional methods (Yermanos, 1979). Furthermore, there are other horticultural limitations since, only a few cuttings can be obtained besides, the hardened terminal shoots are taken during a particular period of the year.

Several attempts have been made to develop tissue culture methods for propagation of *Simmondsia chinensis* **(Aragao, 1977),** but no success could be achieved in transferring the *in vitro* – regenerated plants to soil.The transplanting of *in vitro* rooted shoots of jojoba in recent pulplication is very scarce. We propose this study to develop a protocol for cloning *Simmondsia chinensis,* through tissue culture and successful transplantation of the *in vitro* – raised plants to soil.

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

The prepared explants were cultured on three different 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) then solidified by using 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; 16 hours artificial light (fluorescent light at 30 µM/ hz /sc) and 8 hours darkness.

The investigated treatments in this study which representative of the differential 18 combinations between 2 explant types (shoot tip and nodal cutting) x 3 media type (MS, B_5 & WP) X 3 media strength (full, ½ and ¼) were arranged in a factorial experiment using the complete randomized

design with three replications per each treatment .Every replicate was represented by 10 jars each contained 4 cultured explants.

After four weeks from culturing and incubation, data on survival % of cultured explants, shoot length and number of leaflets / shoot in response to investigated treatments (18 combinations) were recorded.

2. Proliferation "shoot multiplication" stage:

Proliferated shoots throughout the previous stage i.e. establishment " 1st stage " were used for the multiplication stage. Hence, regenerated shoots of both shoot tip and nodal cutting were collected and cultured preliminary on solid Murashege and Skoog (MS), Gamborg (B_5) and Woody plant (WP) media supplemented with several growth regulators i.e., combinations of the cytokinin with auxin, (0.1mg/L) IBA, (30gm/L) sucrose and one of 3 cytokinin kinds i.e., kinetin; BA (benzyl adenine) or 2IP (isopentel adenin) 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.

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

3. Rooting stage:

proliferated shoots were taken and separated from each other under aseptic conditions and cultured on half-strength Murashege & Skoog (MS), Gamborg (B_5) and Woody plant (WP) media supplemented with (30 g/L) sucrose and (7 g/L) purified Bacto - Difico agar with activated charcoal (1 g/L) or without. Rooting media were also varied pertaining auxin treatments i.e., IBA 7 mg + NAA 1 mg/L + Caffiec acid at either 1.0 or 1.5 mg/L, pH was adjusted at (5.6-5.8) and the media were autoclaved. Elongated shoots were transferred to jars containing (40 ml) of the abovementioned rooting media and incubated for one week in the dark and for 3 weeks in light. Where, rooting%; number of rootlets per plantlet and average length of each were recorded in response to the investigated treatments which were representative of 12 combinations between (3media types x 2 activated charcoal x 2 caffiec acid levels) with 3 replications.

4. Acclimatization stage:

Produced Jojoba plantlets were washed with tap water (Ebida, 1991 and Fassuliotis and Nelson, 1992) then dipped in Rhizolix solution (1.0 g/L) as fungicide for (10 min) prior to 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 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 (Smith, 1981). Plantlets were irrigated with half strength (MS, B_5 and WP) maintenance medium (free hormone medium) during the period of hardining (Ebida, 1991). The irrigation was applied depending on the requirement of plantlets. Pests and disease control program was followed as recommended.

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 Duncan's multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

1-Establishment stage:

1-1 Survival percentage:

Concerning the specific effect of different factors involved in this study i.e., explant type, media strength and media type on survival percentage data presented in Table (1) showed that shoot tip recorded higher value of survival percentage than nodal cuttings during both seasons of study.

As for the specific effect of media strength, Table (1) reveals that, full strength media was superior as it exhibited statistically the highest value of survival %, followed in a descending order by half strength media which ranked the second, while quarter strength media ranked last to represent the inferior strength during 2002 and 2003 seasons.

Regarding the specific effect of media type, data obtained revealed, the superiority of (MS) medium over the other ones which showed higher survival % Moreover, (B_5) medium ranked statistically 2^{nd} while (WP) medium ranked 3^{rd} during 1^{st} and 2^{nd} seasons.

Concerning the interaction effect of various combinations on survival %, data obtained revealed that combinations representing cultured shoot tips or nodal cutting on full strength of 3 media (especially MS) and to great exetent shoot tip on half MS strength exhibited statistically the highest survival % during two seasons of study.

On the contrary, cultured explants (shoot tip & nodal cutting) on one fourth WP medium exhibited the least survival % during both seasons of study.

Moreover, other combinations were in between the above-mentioned two extremes. These results go in line with Turk *et al.*, (1992); Zaman *et al.*, (1998) and Silva *et al.*, (2003).

Table (1): Specific and interaction effects of explant type, media strength, media type and their combinations on survival % of jojoba *Simmondsia chinensis* during establishment stage (2002 & 2003 seasons).

Explant type	Media strength / Media type	Strength of media			Mean*	Strength of media			Mean*
		Full	Half	Quarter		Full	Half	Quarter	
		2002				2003			
Shoot tip	B5	82.67ab	78.00c	71.00f	77.38A	82.33ab	77.98cd	68.67f	76.86A
	MS	83.20a	81.67ab	78.20c		83.00a	81.60ab	76.67d	
	WP	81.65ab	73.67e	66.33g		82.25ab	73.60e	66.67g	
Nodal cutting	B5	82.30ab	77.00cd	70.00f	76.76B	82.00ab	76.67d	69.00f	76.16B
	MS	82.35ab	81.50b	76.00d		81.33ab	79.00c	75.00e	
	WP	81.20b	74.20e	66.30g		82.50ab	74.00e	66.30g	
Mean **		82.24A	77.67B	71.31C		82.01A	77.14B	74.89C	
Mean ***		B5	MS	WP		B5	MS	WP	
		76.83B	80.49A	73.89C		76.11B	79.63A	73.80C	

*,**,*** Refer to specific effect of explant type, media strength and media type treatments, respectively. Capital and small letter / swere 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- Length and number of leaves per jojoba explant:

In this regard specific effect of three studied factors i.e., explant type (shoot tip & nodal cutting), media strength (full, half and quarter) and media type (B_5, MS and WP); as well as their possible combinations were investigated pertaining the response of average shoot length and number of leaflets per each.

Referring the specific effect of explant type, it is quite clear as shown from Table (2), that shoot tip had higher values of both shoot length and number of leaflets/shoot than nodal cutting during the two seasons of study.

As for the specific effect of media strength, data displayed that full strength induced statistically the tallest shoot with highest number of leaflets/shoot followed in descending order by half strength and quarter strength, whereas differences were significant during the 2002 and 2003 experimental seasons .

With regard to the specific effect of media type, the results show that (MS) medium proved to be the best medium in establishment stage which exhibited the highest values of both shoot length (cm.) and number of leaflets/shoot while (WP) medium was the least effective during the two seasons of study.

Referring the interaction effect: Table (2) and photo (1) & (2) show, an obvious variances between combinations of explant type; media strength and media type.

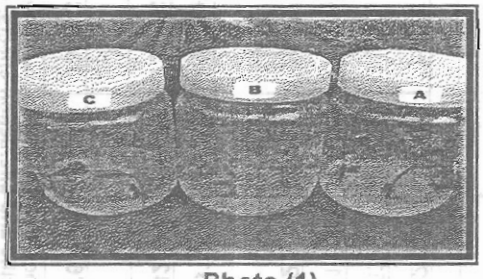

Photo (1)

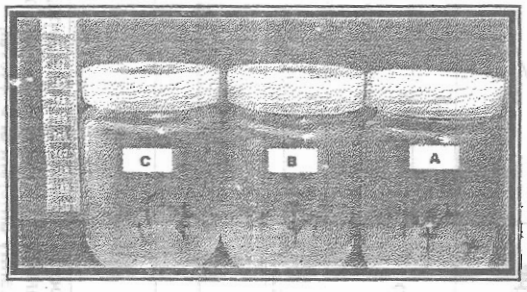

Photo. (2)

Photos. (1 & 2): Effect of explant type, media type and media strength on measurements of establishment stage of jojoba *Simmondsia chinensis* explant :

Photo 1- Shoot tip in full strength media (A: MS, B: B_5, C: WP)

Photo 2- Nodal cutting in full strength media (A: MS, B: B_5, C: WP)

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

Explant type	Media strength / Media type	Shoot length (cm.)			Mean*	No. of leaflets/shoot			Mean*
		Full	Half	Quarter		Full	Half	Quarter	
2002									
Shoot tip	B5	2.08b	1.78c	1.45fg	**1.81A**	5.00c	4.50f	3.75i	**4.48A**
	MS	2.45a	2.01b	1.80c		5.75a	4.58ef	4.00h	
	WP	1.81c	1.58de	1.30hi		4.73de	4.45f	3.58ij	
nodal cutting	B5	1.96b	1.73cd	1.39gh	**1.72B**	4.83cd	4.25g	3.50j	**4.31B**
	MS	2.34a	2.02b	1.65d		5.33b	4.75de	3.75i	
	WP	1.68cd	1.52ef	1.23i		4.58ef	4.41fg	3.41j	
Mean **		**2.05A**	**1.77B**	**1.47C**		**5.04A**	**4.49B**	**3.67C**	
Mean ***		B5	MS	WP		B5	MS	WP	
		1.73B	**2.05A**	**1.52C**		**4.31B**	**4.69A**	**4.19C**	
2003									
Shoot tip	B5	2.07b	1.77ef	1.40j	**1.79A**	5.20b	4.65bc	3.8ef	**4.57A**
	MS	2.43a	2.02bc	1.77ef		5.92a	4.70bc	4.15d-f	
	WP	1.80de	1.57hi	1.25k		4.6bc	4.30cd	3.75ef	
Nodal cutting	B5	1.92cd	1.72e-g	1.38j	**1.70B**	4.67bc	4.33cd	3.58f	**4.28B**
	MS	2.32a	1.96bc	1.60g-l		5.76a	4.42cd	3.83ef	
	WP	1.65f-h	1.50ij	1.20k		4.30cd	4.08de	3.55f	
Mean **		**2.03A**	**1.76B**	**1.43C**		**5.07A**	**4.41B**	**3.78C**	
Mean ***		B5	MS	WP		B5	MS	WP	
		1.71B	**2.02A**	**1.49C**		**4.37B**	**4.80A**	**4.10C**	

*,**,*** 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.

Whereas, the full strength (MS) medium was more effective and showed the highest value of shoot length and greatest number of leaflets/shoot. Conversely, both shoot tip and nodal cutting which cultured on (WP) quarter medium strength gave shortest shoots with lowest number of leaflets/shoot followed in an increasing order by both shoot tip and nodal cutting which cultured on (B_5) quarter medium strength ranked second for number of leaflets/shoot during 2002 and 2003 experimental seasons.

The obtained results go in line with the findings of Tabachnik & Kester (1977), Hammerschlage (1982) and Saker *et al.*, (1999).

2- Multiplication stage:

In this respect specific effect of three studied factors i.e., media type (B_5, MS & WP); cytokinins kind (BA; 2IP and kinetin) and applied concentrations (2, 4, 6 mg/L) of these three cytokinins, as well as their possible combinations were investigated pertaining the response of number of proliferated shoots. Data obtained four weeks after 1^{st}, 2^{nd} and 3^{rd} subcultures of multiplication stage 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 developed shootlets) was resulted followed in a descending order by Gamborg (B_5) medium and Woody plant (WP) medium during two seasons.

With regard to the specific effect of concentration of added cytokinins, Table (3) shows that, 2 mg/L resulted significantly in the greatest number of developed shoots descendingly followed by 4 mg/L and 6 mg/L. Differences were significant during both 2002 and 2003 experimental seasons.

Referring the specific effect of cytokinins kind it is so clear to be noticed that BA (benzyl adenine) exhibited significantly the highest value for number of proliferated shoots descendingly followed by 2iP (isopentel adenine) while Kinetin ranked the last. Differences were significant during both seasons.

Concerning the interaction effect: Data obtained during both 2002 and 2003 experimental seasons as shown from Table (3) and photo (3) displayed that the highest number of developed shoots were significantly in close relationship with Murashige & Skoog (MS) medium supplemented with BA at (2 mg/L). On the contrary, the least values for the number of developed shoots where coupled with Woody plant (WP) medium supplemented with Kinetin at (6 mg/L) during the study. However, other combinations were in between the previously mentioned two extremes during three subcultures.
These results are in general agreement with those found by Liorente *et al.*, (1998), Chitra & Pandmaja (1999), Join & Bubbar (2000) and Erig & Schuch (2003).

Table (3): Specific and interaction effects of media type; growth regulators(kind & concentration) and 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		6.30c	4.67f	2.29kl		7.00c	4.67f	2.90j	
	2iP	4.17ef	3.65g	2.00k	**2.92B**	4.66f	3.33h	2.16lm	**3.30B**	4.64f	4.00g	2.58l	**3.70B**
	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		7.67a	5.67d	2.75i		7.67a	5.67d	3.33i	
	2iP	6.67b	4.33e	2.25j	**3.93A**	6.67b	5.00e	2.50jk	**4.09A**	7.33b	5.00e	2.92j	**4.44A**
	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		5.45d	3.30h	2.09m		5.60d	3.67h	2.33m	
	2iP	3.60g	2.60i	1.72l	**2.48C**	4.33g	3.28h	2.00m	**2.95C**	5.00e	3.33i	2.17mn	**3.29C**
	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		6.00bc	4.00hi	2.32k-m		6.67c	4.66f	3.00jk	
	2iP	4.31d	3.50e	2.15g-l	**3.00B**	4.60fg	3.67i	2.15l-n	**3.241B**	4.60f	3.67gh	2.67k-m	**3.66B**
	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		7.66a	5.66cd	2.67jk		8.33a	5.83d	3.17i-k	
	2iP	6.66b	4.50d	2.20g-i	**3.88A**	6.33b	5.10ef	2.33k-m	**4.037A**	7.66b	5.33e	2.91j-l	**4.62A**
	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		5.33de	3.67i	1.92m-o		5.50de	3.83g	2.42l-n	
	2iP	3.62e	2.75f	1.71ij	**2.600C**	4.30gh	3.00j	2.12l-n	**2.972C**	4.55f	3.50g-l	2.30mn	**3.313C**
	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**	

Table (4): Specific and interaction effects of media type; activated charcoal ; auxin treatments added to one half strength rooting medium and their combinations on rooting percentage ; number of rootlets and average root length (cm.) through rooting stage of jojoba *Simmondsia chinensis* during (2002 & 2003 seasons).

Treatments			Parameters							
Media type	Auxins	Charcoal	Rooting percentage				Number of roots				Root length (cm.)
			A.Ch.		Mean*	Mean**	A.Ch.		Mean*	Mean**	A.Ch.
			With	Without			With	Without			With
2002											
B5	IBA7ML + NAA 1ML + caffeic acid 1 ML		18.00e	17.33f	16.92C	IBA7ML + NAA 1ML + caffeic acid 1 ML	3.15e	2.67f	2.81C	IBA7ML + NAA 1ML + caffeic acid 1 ML	10.67cd
	IBA7ML + NAA 1ML + caffeic acid 1.5 ML		16.67g	15.67h			2.83f	2.58f			8.67ef
MS	IBA7ML + NAA 1ML + caffeic acid 1 ML		20.33e	19.33d	18.75B	20.31A	3.67d	3.29e	3.26B	3.03A	11.00c
	IBA7ML + NAA 1ML + caffeic acid 1.5 ML		18.33e	17.00fg		IBA7ML + NAA 1ML + caffeic acid 1 .5ML	3.28e	2.80f		IBA7ML + NAA 1ML + caffeic acid 1 .5ML	9.67c
WPM	IBA7ML + NAA 1ML + caffeic acid 1 ML		24.20a	22.67b	21.44A		4.67a	4.30b	4.10A		12.00a
	IBA7ML + NAA 1ML + caffeic acid 1.5 ML		19.53d	19.50d		17.17B	4.00c	3.42e		3.15B	9.31d
Mean**			19.53A	18.53B			3.60A	3.18B			10.22A
2003											
B5	IBA7ML + NAA 1ML + caffeic acid 1 ML		21.33c	17.20h	17.95C	IBA7ML + NAA 1ML + caffeic acid 1 ML	3.10e	2.60f	2.65C	IBA7ML + NAA 1ML + caffeic acid 1 ML	10.60c
	IBA7ML + NAA 1ML + caffeic acid 1.5 ML		17.67g	15.60i			2.55f	2.33g			8.60ef
MS	IBA7ML + NAA 1ML + caffeic acid 1 ML		22.00b	19.30e	19.15B	21.03A	3.40d	3.33d	3.14B	3.55A	11.10b
	IBA7ML + NAA 1ML + caffeic acid 1.5 ML		18.00f	17.30gh		IBA7ML + NAA 1ML + caffeic acid 1 .5ML	3.17e	2.66f		IBA7ML + NAA 1ML + caffeic acid 1 .5ML	9.66d
WPM	IBA7ML + NAA 1ML + caffeic acid 1 ML		24.00a	22.33b	21.48A		4.50a	4.33b	3.85A		12.20a
	IBA7ML + NAA 1ML + caffeic acid 1.5 ML		20.00d	19.60de		18.03B	3.58c	2.99f		2.82B	9.30cd
Mean***			20.50A	18.56B			3.38A	3.04B			10.24A

*,**and*** Refer to specific effect of media type; auxin treatment and activated charcoal added to rooting media, 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.

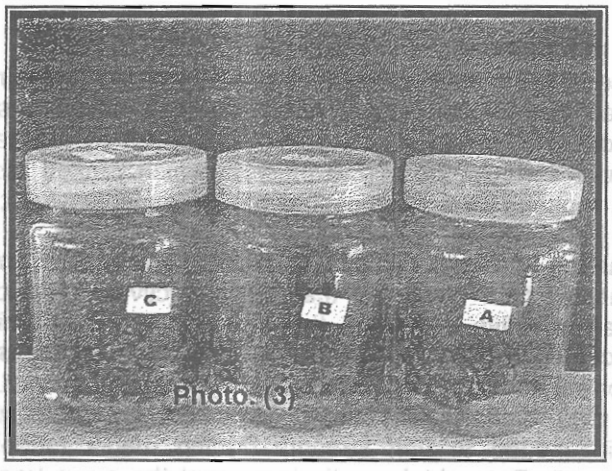

Photo. (3)

Photo. (3): Effect of cytokinins added at 3 levels (2, 4 and 6 mg/L) to three media through the 3rd subculture within multiplication stage of jojoba *(Simmondsia chinensis).*

A: Cultured explant in MS medium supplemented with (2mg /L) BA
B: Cultured explant in B5 medium supplemented with (2mg /L) BA
C: Cultured explant in WP medium supplemented with (2mg /L) BA

3- Rooting stage:

In this regard, adding auxins IBA (7 mg/L); NAA at 1 mg/L and Caffeic acid at (1 or 1.5 mg/L) to half strength B_5, MS & WP media either supplemented with (1.0 g/L) activated charcoal or not in combination were investigated after incubation for 4 weeks through rooting stage (either dark was applied at the 1st week or not) regarding the influence on rooting percentage, number of developed rootlets per plantlet and average root length (cm.) of Jojoba plant. Data obtained are presented in Table (4) and Photo. (4).

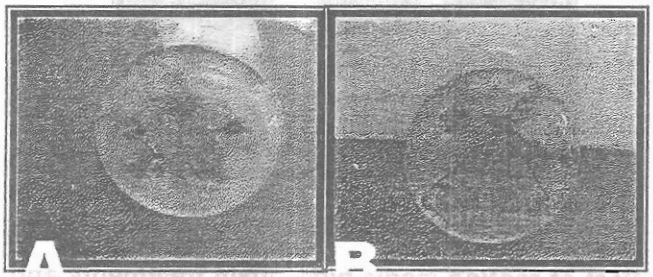

Photo. (4)

Photo. (4): Effect of combinations between media type, auxin treatments and charcoal adding on some measurements during rooting stage of jojoba (*Simmondsia chinensis*)

A: Cultured shootlets in 1/ 2 strength WP + IBA (7.0mg / L) + (1.0mg /L) NAA + (1.0mg /L) Caffeic acid without activated charcoal.
B: Cultured shootlets in 1/ 2 strength MS + IBA (7.0mg / L) + (1.0mg /L) NAA + (1.0mg /L) Caffeic acid without activated charcoal.

3. Rooting percentage:

Concerning the specific effect of media type on the rooting percentage, data showed that, Woody plant (WP) medium exhibited statistically the greatest rooting %, followed in a descending order by Murashige & Skoog (MS) medium and Gamborg (B_5) medium which ranked last. Differences during both seasons were significant as the three media were compared each other.

Regarding the specific effect of adding activated charcoal to half strength media, data displayed that adding activated charcoal to rooting medium was effective. However, the activated charcoal omission reduced rooting percentage of jojoba plantlet during 1st and 2nd seasons.

As for the specific effect of two auxin treatments (7 mg/L IBA + 1 mg/L NAA +1.0 mg/L Caffeic acid) and (7 mg/L IBA + 1 mg/L NAA +1.5 mg/L Caffeic acid) added to half strength rooting media (supplemented with charcoal or not). Data obtained displayed that the auxin treatment with the lower Caffeic acid level (1 mg/L) was more suitable than the higher Caffeic acid rate (1.5 mg/L) during the two seasons of study.

Concerning the interaction effect, it could be safely concluded that half strength (WP) rooting medium supplemented with activated charcoal (1.0 g/L) plus IBA (7 mg/L) + NAA at (1 mg/L) + Caffeic acid at (1 mg/L) gained statistically the highest rooting % when subjected to darkness through 1st week of incubation during the two seasons of study. Moreover, incubation of jojoba plantlets in half strength charcoal omitted (B_5) medium supplemented with IBA at (7 mg/L) + NAA at (1 mg/L) + Caffeic acid at (1.5 mg/L) had the lowest value of rooting % during 2002 and 2003 seasons. In addition, other combinations were in between.

These results are in general agreement with the findings of, Magyar *et al.*, (2001); Thomas, (2003) and Soliman (2004).

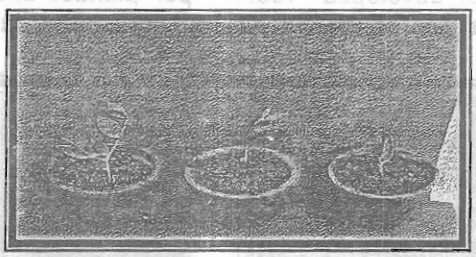

Photo (5)

Photo (5): Effect of rooting media type, auxin treatments and activated charcoal during rooting stage on some measurements of jojoba (*Simmondsia chinensis*) after acclimatization stage:
A: Rooted plantlets on 1/ 2 strength WP + IBA (7.0 mg / L) + (1.0 mg /L) NAA + (1.0 mg /L) Caffeic acid + (1.0g/L) activated charcoal
B: Rooted plantlets on 1/ 2 strength MS + IBA (7.0 mg / L) + (1.0 mg /L) NAA + (1.0 mg /L) Caffeic acid + (1.0g/L) activated charcoal
C: Rooted plantlets on 1/ 2 strength B5 + IBA (7.0 mg / L) + (1.0 mg /L) NAA + (1.0 mg /L) Caffeic acid + (1.0g/L) activated charcoal

3.2- Number of roots per plantlet and root length (cm):

Regarding the response of number of the developed rootlets and their average length to the specific effect of investigated factor i.e, media type; auxin treatments and activated charcoal added through rooting stage, Table (5) displays that the greatest number of roots per plantlet and tallest roots were detected by rooted shortest on (WP) medium followed in a descending order by those on (MS) medium while (B5) medium had statistically the lowest values in this concern during two seasons of study.

As for the influence of adding activated charcoal at (1 gm/L) to half strength medium, data revealed that, the number of roots per plantlet and root length were significantly depressed on the charcoal omitted as compared to analogous one supplemented with charcoal.

As for the specific effect of auxin treatments; data obtained displayed that IBA at (7 mg/L) + NAA at (1 mg/L) + (1 mg/L), Caffeic acid treatment significantly increased the number of roots/plantlet and root length as compared with IBA at 7 mg/L + NAA at 1 mg/L + Caffeic acid at 1.5 mg/L during 2002 & 2003 seasons.

Concerning the interaction effect of various combinations between media type x auxin treatments x charcoal added), Table (5) and photo (4) show that half strength (WP) medium supplemented with (7 mg/L) IBA + (1 mg/L) NAA + (1 mg/L), Caffeic acid and activated charcoal gave significantly the greatest number and tallest rootlets per plantlet. On the contrary, adding IBA at (7 mg/L) + NAA at (1 mg/L) + Caffeic acid at (1.5 mg/L) without activated charcoal to half strength (B_5) medium gave the lowest number of rootlets/plantlet and shortest rootlets during the two seasons of study.

In addition, other combinations were in between as compared to the previously mentioned two extents.

These results are in general agreement with the findings previously mentioned by Ishida *et al.*, (1989); Vasar *et al.*, (2000) and Soliman (2004). However, the presence of activated charcoal in rooting medium was in general agreement with the findings of Bondok *et al.*, (1989); Fouad *et al.*, (1995) and Soliman (2004).

4 - Acclimatization stage:

In this stage:- The plantlets produced from the best treatments (rooting media X auxins and charcoal added) through the previous stage (rooting) were chosen and cultivated on transplanting medium consisting of (vermiculite: peat moss: sand) at (1:1:1) ratio by volume for acclimatization stage.

Table (5) shows the effect of some Specific treatments used in rooting stage on survival% and some growth parameters (shoot length and number of leaves) during acclimatization stage.

Rooted plantlets in half strength WPM + IBA at 7mg/L+ NAA at 1mg/L+ Caffiec acid at 1.0 mg/L + 1.0 mg/L activated charcoal gave the highest survival%, tallest shoots and higher number of leaves followed in descending order by rooted plantlets in half strength MS medium + IBA at 7mg/L+ NAA at 1mg/L+ Caffiec acid at 1.0 mg/L + 1.0 mg/L activated charcoal. While rooted plantlets in half strength B5 medium + IBA at 7mg/L+ NAA at 1 mg/L+ Caffiec

acid at 1.0 mg/L + 1.0 g/L activated charcoal had the least values during the two seasons of study.

These results are in general agreement with the finding of Hoffmann *et al.*, (1999); Benzioni *et al.*, (2003) and Soliman (2004).

Table (5): Comparison between the most effective three rooting treatments (rooting medium X auxins and charcoal added) on survival %; shoot length (cm) and number of leaves of acclimatized newly regenerated jojoba plantlets during 2002 and 2003 seasons.

Parameters	Survival		Shoot length		No. leaves	
Treatments	2002	2003	2002	2003	2002	2003
½ strength WP +IBA(7 mg/L) + NAA (1 mg/L) + Caffeic acid (1 mg/L) + A. C.1g/L	76.33 a	76.00 a	10.00 a	10.20 a	11.00 a	11.20 a
½ strength MS +IBA(7 mg/L) + NAA (1 mg/L) + Caffeic acid (1 mg/L) + A. C.1g/L	74.33 b	74.30 b	9.20 b	9.00 b	10.00 b	10.25 b
½ strength B5 +IBA(7 mg/L) + NAA (1 mg/L) + Caffeic acid (1 mg/L) + A. C.1g/L	71.33 c	71.60 c	7.67 c	7.66 c	9.20 c	8.83 c

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الإكثار بتقنية زراعة الأنسجة لبعض أنواع الفاكهة:
ب- إكثار الهوهوبا بتقنية زراعة الأنسجة

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أجريت هذه الدراسة في موسمي ٢٠٠٢ ، ٢٠٠٣ بمعمل زراعة الأنسجة بمعهد بحوث البساتين على نبات الهوهوبا بهدف تطوير إكثارها بطريقة زراعة الأنسجة لصعوبة إكثارها بالطرق التقليدية ويستلزم ان تجهز العقل في فترة معينة ومحدودة بالإضافة إلى ذلك فإن نسبة نجاحها قليلة وعلى ذلك فقد تم تجريب عدة معاملات خلال المراحل التالية (الأساس ـ التضاعف ـ التجذير والأقلمة) وقد تم دراستها كالآتي:ـ

أولا: مرحلة الأساس:

في هذه المرحلة أجريت تجربة عاملية لدراسة التأثير النوعي لكل من المنفصل النباتي (البرعم الطرفي والعقلة ذات البرعم الواحد) وكذلك نوع البيئة (WPM , MS , B_5) وتركيز أملاحها الأساسية (كاملة ونصف وربع تركيز على نسبة البقاء ومتوسط طول الفريخات وعدد الوريقات المتكونة عليها) فبعد إجراء التعقيم للمنفصلات النباتية ثم زراعتها على البيئات الغذائية السابقة الذكر مضاف إلى كل منها ٠,١ ملجم/ لتر إندول حامض البيوتيريك ١ ملجم/ لتر بنزيل أدنين وبعد أربعة أسابيع من الزراعة أظهرت النتائج المتحصل عليها الآتي:

- تفوق البرعم الطرفى على العقلة ذات البرعم الواحد فى تسجيل أعلى نسبة بقاء وكذلك طول الفريخات وعدد الأوراق المتكونة عليها.
- أثبتت بيئة موراشيج وسكوج كاملة القوة أنها أفضل البيئات فى تسجيل أعلى القيم للقياسات الثلاثة السابقة الذكر وكان العكس صحيحا مع العقلة ذات البرعم الواحد والتى تم زراعتها على بيئة الأشجار الخشبية ذات الربع تركيز .

ثانيا : مرحلة التضاعف:

فى هذه المرحلة تم إعادة الزراعة للفريخات الناتجة من مرحلة الأساس على نفس البيئات الغذائية السابقة الذكر ذات القوة الكاملة المضاف إليها ثلاثة أنواع من السيتوكينينات (بنزيل أدنين وأيزوبنتيل أدنين وكينيتين) كل بثلاث تركيزات هى (٢،٤،٦ ملجم / لتر) فى تباديل وتراكيب مختلفة بينها لدراسة تأثيرها على عدد الأفرخ المتكونة وقد أوضحت الدراسة النتائج التالية :ـــ

- تفوقت بيئة موراشيج وسكوج (MS) فى زيادة عدد الفريخات الحديثة المتكونة على بيئتى جامبورج وبيئة الأشجار الخشبية .
- البنزيل أدنين بتركيز ٢ ملجم/لتر كان أكثر تفوقا فى هذا الشأن .
- إضافة الكينيتين بتركيز ٦ ملجم/ لتر على بيئتى جامبورج أو الأشجار الخشبية أعطى أقل عدد تفرعات وهذا خلال الثلاث نقلات (subculture).

ثالثا: مرحلة التجذير:

تم تجذير الأفرخ الجديدة المتكونة فى مرحلة التضاعف على البيئات الثلاثة السابقة الذكر ذات النصف تركيز لأملاحها والمحتوية على الفحم النشط بتركيز ١ جم / لتر أو الخالية من الفحم النشط والمضاف إليها ٧ ملجم / لتر اندول حامض البيوتيريك (IBA) + نفثالين حامض الخليك بتركيز (١ مجم / لتر) + حامض الكافييك بتركيز (١ أو ١,٥ مجم / لتر) .

فقد درس تاثير التفاعل للتراكيب المختلفة على إستجابة قياسات التجذير الثلاثة (نسبة التجذير ــ عدد الجذور المتكونة ــ متوسط طول الجذور) وقد أوضحت الدراسة النتائج التالية : ــ

- سجلت بيئة الأشجار الخشبية (WPM) أعلى قيمها لنسبة التجذير وعدد الجذور المتكونة ومتوسط طول الجذر.
- إضافة الفحم النشط (١جم/لتر)إلى البيئات أدى إلى زيادة معنوية للقياسات الثلاثة خلال موسمى الدراسة .
- إضافة ٧ مجم/لتر إندول حامض البيوتيريك + ١ ملجم / لتر نفثالين حامض الخليك + ١ مجم / لتر حامض الكافييك أعطى أعلى قيم لمقاييس التجذير الثلاثة السابقة الذكر، وكان العكس صحيحا مع بيئة جامبورج (B_5) الخالية من الفحم النشط والمضاف إليها ٧ ملجم/ لتر إندول بيوتيريك (IBA) + ١ ملجم/ لتر نفثالين حامض الخليك (NAA) + ١,٥ ملجم/ لتر حامض الكافييك خلال موسمى الدراسة

رابعا: مرحلة الأقلمة:

في هذه المرحلة أجريت تحت ظروف الصوبة الزجاجية حيث تم نقل نباتات الهوهوبا الناتجة من أفضل معاملة لكل بيئة من البيئات الثلاثة (B_5 , WPM , MS) المستخدمة تحت الدراسة فى مرحلة التجذير لأقلمتها وذلك بغسلها بماء الصنبور وغمسها في محلول ريزولكس ثم تفريدها 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 : ــ

- النباتات المجدرة على بيئة الأشجار نصف تركيز والمحتوية على ٧ ملجم / لتر إندول حامض البيوتيريك + ١ مجم / لتر نفثالين حامض الخليك + ١ مجم / لتر حامض الكافييك + ١ جم فحم نشط كانت الاكثر تفوقا بالنسبة لمقاييس الاقلمة الثلاثة (نسبة البقاء ـــ طول النبات وعدد الأوراق) .
- النباتات المجدرة على بيئة B_5 نصف تركيز والمضاف إليها ٧ مجم /لتر اندول حامض البيوتيريك(IBA) + ١ ملجم نفثالين حامض الخليك (NAA) + ١ ملجم حامض الكافييك + ١ جم فحم نشط أظهرت أقل قيم في هذا الصدد .