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EFFECTIVE PROTOCOL FOR *in vitro* SHOOT PRODUCTION THROUGH FLOWER BUD EXPLANTS OF JOJOBA *(SIMMONDSIA CHINENSIS)*

S.A.M. Hassan[1,2], Nagwa S. Zaied[1,2*], H.S.A. Hassan[2] and Dorria M.M. Ahmed[2]

[1]Tissue Culture Technique Lab, Central Laboratories Network, National Research Centre, 33 El Buhouth St., Dokki, Giza, Egypt.
[2*]Pomology Dep., National Research Centre, 33 El Buhouth St., Dokki, Giza, Egypt.

Abstract

Flower buds (male and female explants) from Jojoba were cultured individually on MS medium supplemented with 0.2 mg/l 6-benzylamine (BAP), 30g/L sucrose and 7g/L Difico Bacto agar. The establishment stage showed that the best sterilizing agent, concentration for surfaced is infection of explants was Clorox at (10 and 20% for 15 min) and mercuric chloride ($HgCl_2$) at (0.05 and 0.1% for 10 min) different antioxidant such as citric acid, ascorbic acid and polyvinylpyrolidone (PVP) were tested. Different concentrations of 6-benzylaminopurine (BA) at the rates of (0.0, 1.0, 2.0, 3.0 and 4.0 mg/L) and 2, 4-dichlorophenoxyacetic acid (2, 4-D) at the same concentrations were including. Data indicated that using mercuric chloride ($HgCl_2$) at 0.1% for 10min gave the highest survival percentage and the lowest contamination for flower buds (male and female explants). However, adding PVP to the culture medium is recommended to achieve the best results for increasing survival percentage and greening and reducing necrosis and brewing of flower bud explants. Meanwhile, higher 2, 4-D concentration (4.0 mg/L) enhanced callus production. On the other hand, lower BAP concentration (1.0 mg/L) improved greening and decreased necrosis. In addition, BAP at the rate of 2.0 mg/L enhanced plantlets regeneration of male explants. In general the responses of female explants were higher than those explants from male explants when using the same concentration of most experiment under study.

Key word: BAP, 2, 4-D, Callus Production. Jojoba-Flower Buds.

Introduction

Jojoba (*Simmondsia Chinensis*) a promising dioecious shrub of arid zones is commercially valuable for its seeds. A major problem in seeds production is that Jojoba is dioeciously plant where its sex is not easily determined prior to flowering (3-4 years from cultivation) Tyagi and Prakash 2004 and Mill *et al.,* 2009). Jojoba is clonally propagated by nodes and the rate of propagation is very limited because the nodes are hard to roots, so the only solution to solve this problem is through the rapid mass production by micropropagation technique (Heba Allah *et al.,* 2009 and Taha and Hassan., 2016). Tissue culture of Jojoba offers a promising method for mass production of superior pathogen free clones for commercial plantation (Mills *et al.,* 1997). Propagation of Jojoba plants can be achieved by rooting semi hard wood cuttings but the maximum number of possible propagates is limited by time of planting and plant size (Hackett 1981 and Taha *et al.,* 2016).

Several factors affecting *in vitro* establishment stage of Jojoba such as surface sterilization of explants, Phenolic exudation and its control and plant growth regulator. Contaminated of Jojoba plants was a major problem during initiation of cultures under *in vitro* conditions. The explants were mainly contamination by bacteria and fungus. Surface sterilization gave good explanation for Bambusatulda by using 0.1-0.2% mercuric chloride (Mishra *et al.,* 2008). Adding antioxidants such as ascorbic acid was the most successfully method to reduce Phenolic compound production and browning of apple (Ciccotti *et al.,* 2008). Supplementation of the culture medium with highly cytokinin induced the best

***Author for correspondence :** E-mail: sayed_ebead@yahoo.com

shoot proliferation of Jojoba. On the other hand, callus induction was greatest with aux in alone or in combined with cytokinin Fayek *et al.,* (2007). The aim of the present study was the effective protocol for *in vitro* shoot production through flower buds (male and female explants) of Jojoba.

Materials and Methods

This study was carried out at the tissue culture laboratory of Pomology Department, National Research Center, Dokki, Giza, Egypt during seasons of 2017 and 2018. Flower bud explants (Male and Female) from Jojoba (*Simmondsia Chinensis*) were taken and subjected to running water for 3 minutes to get rid of dirt and free Phenolic compounds. The explant was cultured on MS medium supplemented with 0.5 mg/L BAP (6-benzylaminopurine), 0.1 mg/LIBA (Indole 3-butyric acid), 30g/L sucrose and 7g/L Difico Bacto agar which considered as basal medium. The pH of the media was adjusted to 507 and autoclaved at 12°C and 15Ib/inch^2 for 15 minutes (Mustafa *et al.,* 2016). The cultured explants were incubated under 16 hours of artificial light (Fluorescent light at 30 uM/m^2/sec) and 8 hours of dark at average temperature of 25±3°C. Subculturing was done regularly at 6 weeks intervals in all experiments. Thus, the following experiments were carried out.

Establishment stage

Surface sterilization

The explant was sterilized using solution of commercial bleach (^{66}cloro × 5.25% a available chlorine) at two concentrations 10 and 20% for 15 min. The other sterilizing agent used was mercuric chloride ($HgCl_2$) at 0.05 and 0.1% for lomins. After sterilization, flower buds (Male and Female explants) were directed out and placed immediately on the establishment culture MS medium. After 6 weeks from culturing survival and contamination percentages were recorded.

Table 1: Effect of surface sterilization with sodium hypochlorite (NaOCl) and mercuric chloride ($HgCl_2$) on the survival and contamination percentages of flower buds of Jojoba (male and female explants).

Explant	Male		Female	
Measurement / Sterilization	Survival %	Contamination %	Survival %	Contamination %
10% Clorox	50.63^c	49.61^A	43.50^C	52.67^A
20% Clorox	85.10^B	15.30^B	7.50^B	32.60^B
0.05% $HgCl_2$	91.60^A	10.67^C	84.30^A	15.30 D
0.01% $HgCl_2$	40.30^D	15.00^B	36.23^D	26.00^C

Effect of antioxidant treatment:

The following antioxidant treatments were studied.

1- Control: Explant was immersed in sterilized distilled water for two hours.

2- Antioxidant solution: the explants were immersed for 2 hours in a solution mixture consisted of 100 mg/L ascorbic acid and 150 mg/L citric acid as a pretreatment.

3- Polyvinyl pyrolidone (PVP): 100 mg/L PVP was added to the culture medium.

They were subjected to the antioxidant treatments to determine the most suitable antioxidant treatment that succeeded in reducing or eliminating accumulation of the phenolic compounds and in turn enhanced explant development.

Effect of different concentrations of BAP and 2, 4-D

6- benzylaminopurine (BAP) and 2, 4 dichlorophenoxyacetic acid (2, 4-D) were tested at the level of 0.0, 1.0, 2.0, 3.0 and 4.0 mg/L to the culture medium under study to detect the most effective that maximized callus induction.

1-D- Plantlets regenerations:

Different concentrations of BAP *i.e.,* 0.0, 1.00, 2.00, 3.00 and 4.00 mg/L were added to MS medium to find out the best concentration encourage the highest number of regenerated plantlets and growth parameters of Jojoba flower Buds.

Data and Calculation

Survival %, Callus production %, Contamination %

Table 2: Effect of antioxidant treatments on necrosis, survival percentage browning and greening on *Simmondsia Chinensis* (male and female explants).

Explant Measurement	Male				Female			
	Necrosis (score)	Survival	Browning (Score)	Greening (score)	Necrosis (score)	Survival	Browning (Score)	Greening (score)
(Control)	3.51^A	43.60^C	3.67^A	1.67^C	3.67^A	42.60^C	3.67^A	1.78^C
(Citric+ ascorbic acid)	2.56^B	50.67^B	2.18^C	2.15^B	2.86^B	55.67^B	2.33^C	2.67^B
1% PVP	2.00^C	66.60^A	2.90^B	3.67^A	1.30^C	67.33^A	2.98^B	3.80^A

Control = culture without dipping in antioxidant.

Meanwhile, Scores were given for necrosis, greening (determined as the degree of keeping original explants color and the degree of green leaves), growth and Number of regenerated plantlets. These parameters were expressed as scores at the following rate. Negative results = 1, below average + 2; Average = 3, above average-4 and excellent-5. These scores were recommended by Pottino (1981).

Statistical Analysis

The treatments were arranged in a complete randomized design with five replicates for each treatment, data were subjected to analysis of variance according to Snedecor and Cochron (1980).

Results and Discussion

Sterilization of Explant:

Table 1 show the effect of surface sterilization with sodium hypochlorite (NaOCl) and mercuric chloride ($HgCl_2$) on the survival and contamination percentages on flower buds of Jojoba. It is clear that the most effective concentration of $HgCl_2$ was 0.05% for 10min which gave the highest percentages (91.60 and 84.30%) of survival on male and female explant and the lowest contamination percentage (10.67 and 15.30%) followed by using NaOCl 20% for 10 min then NaOCl 10% for 1 min as compared with $HgCl_2$ at 0.1% for 10 min in a descending order. In general, using 0.05% $HgCl_2$ for 10 min gave the highest survival and the lowest contamination age for flower buds (male and female explants). On the other hand, survival percentages were low and the contamination percentages in flower buds (male and female explants) were higher when the explants were sterilized by 0.1% mercuric chloride ($HgCl_2$) for 10 min. These results partially agreed with the findings of (Shahin 2003). Found that using 0.05% mercuric chloride ($HgCl_2$) for 10 min was successful in surface sterilization and gave good explant in very low visual contamination of mangoes. Also, using 10% sodium hypochlorite and surfactant rots for 10 min was successful in surface sterilization and gave good explanation for grape tissue (Vitisvinifera) (Sim 2006).

Effect of antioxidant treatments:

Table 2 explains the effect of different antioxidant treatments on necrosis, survival percentage, drowning and greening on Jojoba (male and female explants). It is clear that PVP treatment encouraged a significant increase in survival percentage and greening parameters while statistically reduced both necrosis and browning as compared with the other used treatments. Meanwhile, antioxidant pretreatment took the second rank in improving necrosis, survival percentage, Browning and greening. On the other hand, control treatment induced the lowest significant effect on all other treatments. In general, the before mentioned results reflected that adding of PVP to the culture medium is recommended to achieve the results. This may be due to the effect of polyvinylpyrolidone (PVP) in reducing oxidation of Phenolic compounds as compared with the control, which adsorbed the particles of both Phenolic compounds and other compounds in the culture medium, which greatly affect the nutritional status of the cultured explant. These results are in harmony with the findings of (Shahin 2003) reported that addition of antioxidants such as PVP, citric acid and ascorbic acid may prevent the oxidation of phenols. In addition, Hassan (2004) reported that PVP was ineffective at the rate of 100 mg/L in controlling oxidation of Phenolic compounds in apple explants.

Effect of BAP and 2, 4 D at different concent-rations

Data in table 3 and photo 1 indicated that the best callus induction response for both explants (male and female) occurred at 4.0 mg/L 2,4-D followed by 3.0 mg/L 2,4-D then 4.0 mg/LBAP as compared with the other used treatments and the control. However, the response of female explants were higher than those explants from male plant when using the same concentration. On the other hand, the lowest response appeared at 1.0 mg/L 2,4-D or BAP giving a mean value of callus induction. There was a significant increase in the percentage response with increasing 2,4-

Table 3: Effect of different concentrations of BAP and 2,4D on the (%) response of callus induction, necrosis and greening on *Simmondsia Chinensis* (male and female explants).

Explant	Male			Female		
Measurement Concentration	Necrosis (Score)	Callus induction%	Greening (Score)	Necrosis (Score)	Callus induction%	Greening (Score)
0.00	4.63^{A}	17.00^{F}	1.17^{D}	4.30^{A}	19.67^{F}	1.00^{A}
1.0 BAP	1.42^{D}	24.00^{F}	3.67^{A}	1.30^{D}	26.23^{F}	3.33^{A}
2.0 BAP	1.36^{D}	26.00^{E}	3.66^{A}	1.67^{D}	36.00^{D}	3.67^{A}
3.0 BAP	2.53^{C}	40.33^{C}	2.90^{B}	2.50^{C}	43.22^{C}	2.86^{B}
4.0 BAP	3.68^{B}	43.00^{BC}	1.38^{C}	3.67^{B}	49.00^{DC}	1.30^{C}
1.0 2,4 D	1.3^{D}	20.83^{F}	2.90^{B}	1.30^{D}	22.31^{F}	2.67^{B}
2.0 2,4 D	2.67^{C}	26.33^{E}	3.67^{A}	2.80^{C}	38.60^{D}	3.80^{A}
3.0 2,4 D	3.368^{B}	58.60^{B}	1.36^{C}	3.67^{B}	54.22 B	1.63^{C}
4.0 2,4 D	4.60^{A}	67.33^{A}	1.00^{D}	4.00^{A}	76.33^{A}	1.00^{A}

D or BAP concentration up to 4.0 mg/L. Maximum response 76.33% on female explant and 67.33% on male explant with explants treated with 4.0 mg/L 2, 4-D. Also, maximum response percentage induction was recorded in the control. However, lower concentration (1.0 or 2.0 mg/L) of BAP to the culture medium was valuable in significantly induced necrosis and improved greening in comparison with the other concentrations of male and female explants under study. Meanwhile, supplementation the medium with 2.0 mg/L of 2, 4-D significantly improved greening parameter in relation to the others. On the other hand, addition of 1.0 mg/L 2, 4-D to the cultured medium caused significantly a reduction necrosis of male and female explants. In general, callus induction from Jojoba cultured on MS medium supplemented with 4.0 mg/L 2, 4-D. The response of female explants was higher than those explants from male plant when using the same concentration. These results are in harmony with Hussan (2004) recommended 4.0 mg/L BAP for the best callus production of apple rootstock. Also (AL-Obaidi 2006) found that A callus produced from Jojoba (male and female) explants using when explants were cultured on MS medium suing hormone combination of (0.5 mg/L BA, 2.5 mg/L 2, 4-D).

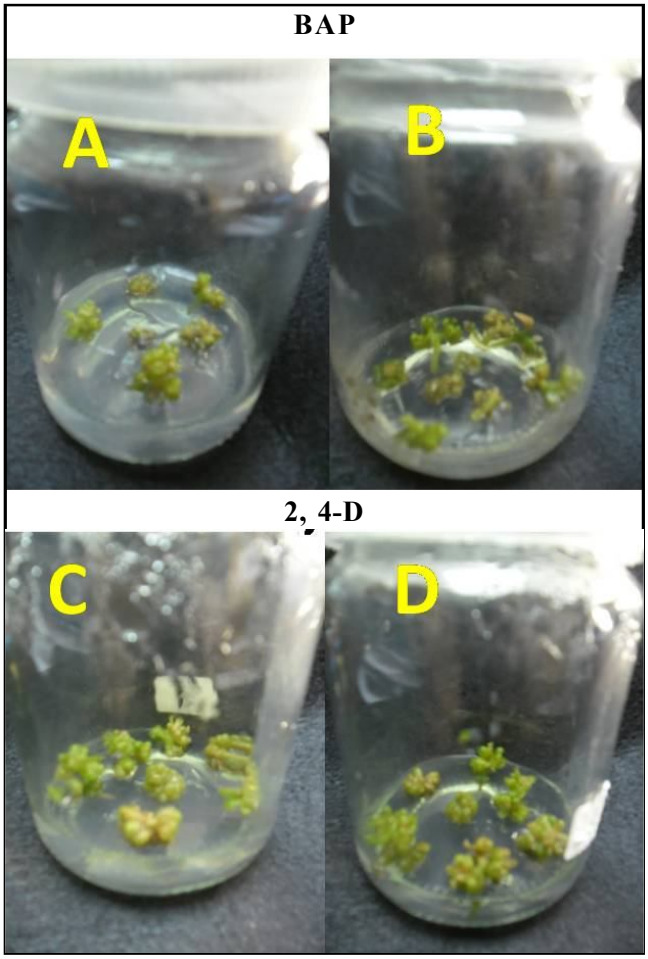

Photo 1: Effect of different concentrations of BAP and 2,4D on the (%) response of callus induction, necrosis and greening on *Simmondsia Chinensis* (male and female explants):
(A): Male (B): Female (C): Male (D): Female

Effect of Hormonal balance:

Data of table 4 and photo 2 reflected the effect of hormonal balance on number of regenerated plantlets and growth parameters of Jojoba (male and female) explants. It is clear that supplement of the culture medium with BAP at 1.0 mg/L maximized growth and greening significantly while only 3.0 mg/L level significantly increased number of regenerated plantlets in relation to the other concentrations under study. Meanwhile, supplemented of the culture medium with 4.0 mg/L BAP it's maximized necrosis significantly. However, growth and greening significantly increased when 0.0 mg/L control or 4.0 mg/L BAP was added to the cultured medium of male and female explants in relation to the other BAP concentrations. On the other hand, necrosis parameter was not statistically affected by different concentrations of BAP. However, regenerated plantlets significantly increased when medium was supplemented with 2.0 mg/L BAP. In general, BAP supplementation at 2.0 mg/L level to the culture medium of female explant resulted in significant maximizing Number of regenerated plantlets compared with other concentrations. Also, supplementation at 3.0 mg/L BAP level to the culture medium of male explant succeeded in increasing number

Table 4: Effect of hormonal balance on No. of regenerated plantlet and growth parameters of *Simmondsia chinessis* (Male and Female explants).

Explant Parameters BAP Concentration	Male				Female			
	Necrosis (score)	Growth	No. of regen-erated planet	Greening (score)	Necrosis (score)	Growth	No. of regen-erated planet	Greening (score)
(Control)	1.67^{C}	4.00^{B}	1.67^{C}	3.20^{B}	1.50^{A}	4.67^{A}	1.30^{D}	4.00^{A}
1.0	1.52^{C}	4.67^{A}	1.53^{CD}	4.50^{A}	1.39^{A}	4.00^{B}	1.68^{C}	3.67^{B}
2.0	1.75^{C}	4.20^{B}	1.32^{D}	4.00^{B}	1.52^{A}	3.33^{C}	4.36^{A}	3.17^{C}
3.00	2.30^{B}	1.30^{C}	3.00^{A}	2.30^{C}	1.60^{A}	2.67^{D}	2.00^{B}	2.00^{D}
4.00	3.50^{A}	1.00^{D}	1.67^{C}	1.23^{D}	1.67^{A}	4.67^{A}	1.20^{D}	4.68^{A}

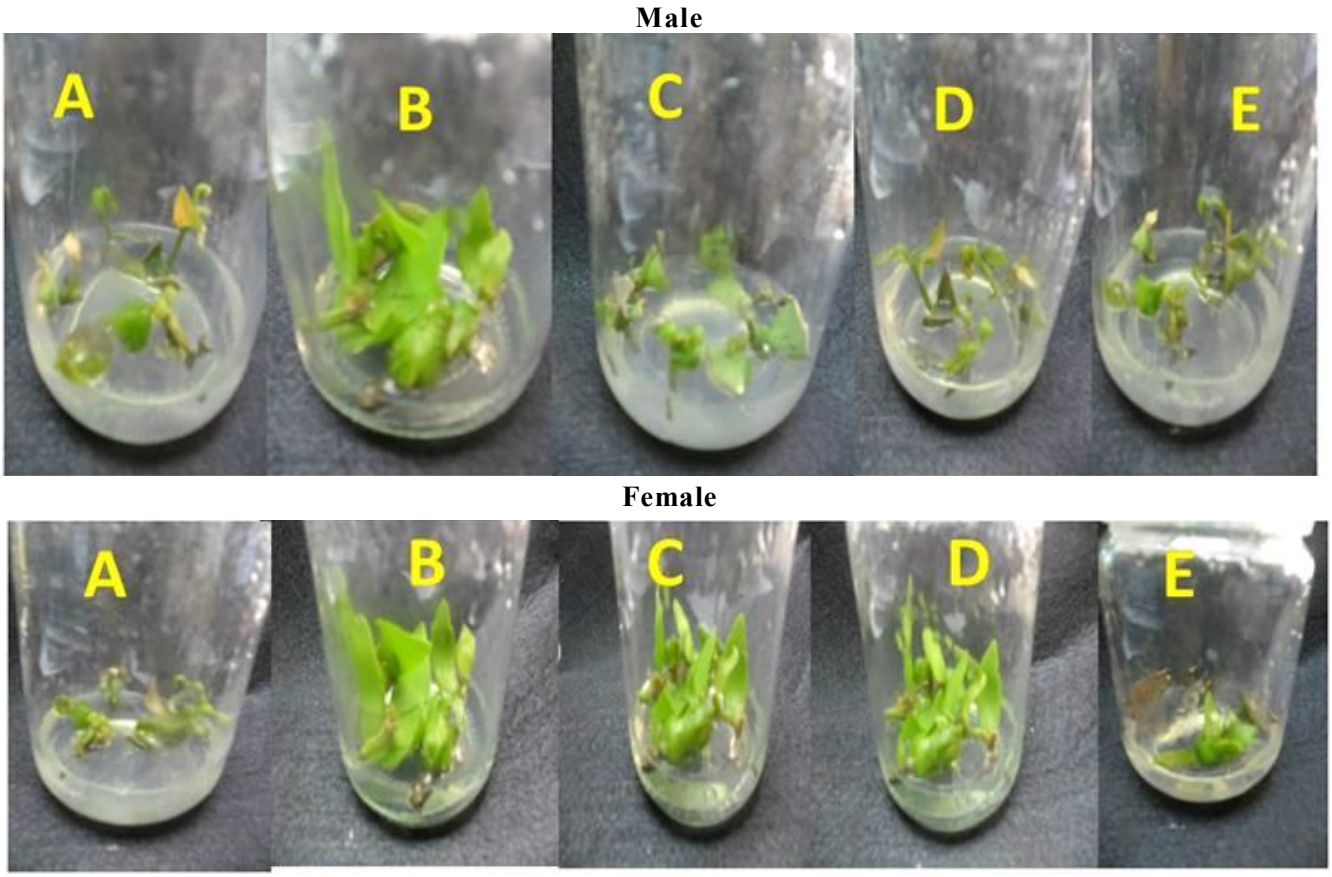

Photo 2: Effect of hormonal balance on No. of regenerated plantlet and growth parameters of *Simmondsia chinessis* (Male and Female explants). (A): control (0.00) (B) 1.0 mg/l BAP (C) 2.0 mg/l BAP (D) 3.0 mg/l BAP (E) 4.0 mg/l BAP

of regenerated plantlets. These results are in general agreement with the findings of (Baiea, 2002) found that the best regeneration observed on date palm cultured on MS medium supplemented with 2.0 and 3.0 mg/L BAP.

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Conclusion

From the current study, it can be concluded that survival percentage of flower buds (male and female explants) from Jojoba was increased significantly by using mercuric chloride ($HgCl_2$) at 0.1 % for 10 min and PVP at 100 mg/l. Maximum callus production was achieved on MS medium fortified with 2, 4-D at 4.0mg/l ,regenerated shoots were multiplied in MS medium supplemented with BAP at 20 mg/l. the responses of female explants were higher than those explants from male explants when using the same concentration of most experiment under study.

References

Al-Obaidi, J.R.J. (2006). Differentiation between male and female of Jojoba plant in vivo and *in vitro* using PCR techniques. Ph-D. Ministry of higher Education and Scientific Research Al-Nahrain University.

Baiea. M.A. (2009). Physiological and cytological studies on tolerance of some stone fruits rootstocks to salinity and drought by using micropropagation. Ph.D. Thesis, Zagazig University, Egypt.

Ciccotti, A.M., C. Bisognin, I. Battoclotti, A. Salvadori, M. Herdemertens and W. Jarausch (2008). Micropropagation of apple proliferation-resistant a ponictic Malus Sieboldii genotypes. *Agron. Res*, **6:** 445-458.

Fayek, M.A., E.A. Shaaban, A.A. El-Obeidy and R.A. Taha (2007). *In vitro* propagation of three femal Jojoba clones (Simmondsis Chinesis (Link.) Schneider) using *in vitro* culture. *World. J. Agric. Sci.*, **6:** 446-450.

Hassan, A.A. (2004). Propagation Improvement of some apple rootstock and passion fruits by using tissue culture techniques. Ph.D. Hort., Fac. Agric. Moshtohor. Zagazig University, Egypt.

Heba Allah, A.M., M.K. El Bahr, Z.M. Adam, H.A. Mouryand and M.El. Solliman (2009). *In vitro* celonal propagation of Jojoba (*Simmondsia Chinensis* (Link) Schra): *Australian*

Journal of Basic and Applied Sciences, **3(4):** 3128-3136.

Hutechinson, J.K. (1984). Factors affecting shoot proliferation and root initiation in organ culture of apple 6 Northem Spy; *Sci. Hortic*, **22:** 347-358.

Mills, D., S. Wenkart and A. Benzioni (1997). Micropropagation of *simmonolsia chinensis* (Jojoba): In: Y.P.S. Bajaj, Editor, Biotechnology in Agricutlure and forestry, High-Tech and Micropropagation VI. Springer Veriag, *Berlin*, **40:** 370-393.

Mills. D.Y. Zhou and A. Benzioni (2009). Effect of substrate, medium composition, irradiance and ventilation on Jojoba Plentlets at the rooting stage of micropropagation. Scienta, 01.021.

Mishra, Y.P., K. Patel, S. Yadav., S. Shirin and S.A. Ansari (2008), A micropropagation system for cloning of Bambusatul-daRoxb. *Scient. Hortic.,* **115:** 315-318.

Mustafa, N.S., S.A.M. Hassan and Rania A. Taha (2016). *In vitro* Studies on Growth and Rooting of Some Fig Cultivars. *Research Journal of Pharmaceutical, Biological and Chemical Sciences.,* **7(5):** 124-130.

Pottino, B.G. (1981). Methods in plant tissue culture. Dept. of Hort. Agric. College, Maryland University, College Park, Maryland USA, 8-29.

Rania A. Taha and S.A.M. Hassan (2016). Studies on silver nitrate impact on jojoba *in vitro* culture. *International Journal of Pharm Tech Research.,* **9(8):** 77-83.

Rania A. Taha, S.A.M. Hassan, Dorria M.M. Ahmed and Nagwa S. Zaied (2016). A Comparative Study on Different Cytokinin Types and Carbon Source Concentrations on *In vitro* Proliferation of Jojoba (*Simmondsia chinesis* Link (Schneider)). *International Journal of ChemTech Research.,* **9(8)**: 178-184.

Shahin, M.F.M. (2003). Studies on vegetative propagation of Mango by using new techniques. Ph.D. Hort., Fac. Agric. Ain Shams University, Egypt.

Sim, S.T. (2006). Virus dimination from grape selection using tissue culture. FPS Grap Program News letter, November, 30-31.

Snedecor, W.B. and G.W. Cochran (1980). Statistical Method 6th Ed. Low State College. Press Amer low, U.S.A.

Tyag, R.K. and S. Prakash (2004). Genotype and Sex specific protocols for *in vitro* Micropropagation and Medium tera conservation of Jojoba. *Biologia Plantarum,* **8(1):** 19-23.