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Banat's Journal of Biotechnology
2015, VI(11)

TISSUE CULTURE OF *Simmondsia chinensis* (Link) SCHNEIDER

DOI: 10.7904/2068–4738–VI(11)–45

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Abstract. Jojoba (*Simmondsia chinensis* (Link) Schneider) a multipurpose and monogeneric dioecious shrub from arid zones, has emerged as a cash crop all over the globe. Its seed propagation poses severe problems due to its male–biased population: the male: female ratio is five to one. To overcome this phenomenon, asexual propagation using vegetative methods such as tissue culture could be used. Investigations were conducted for propagating jojoba plants aseptically *in vitro* staring from nodal segment with two full or half leaves and shoot tip explants. The growth media (MS) were a basal medium additional to Gamborg's vitamins (B5) and then modified MS medium (MMS) supplemented with different plant growth regulators (PGRs). The results indicated that MS–B5 growth medium was better than MMS medium after 40 and 60 days of culture. Shoot tip explants were superior to nodal segments with two full or half leaves at different concentrations and combinations of PGRs. The highest rooting percentage was recorded in MS/2–B5 + 1 mg/L IBA. This procedure produced an efficient protocol for jojoba tissue culture.
Keyword*:* Explant, GA_3, *In vitro*, Jojoba, PGRs

Introduction

Jojoba (*Simmondsia chinensis* (Link) Schneider) commonly known as jojoba, is a perennial shrub belonging to the Simmondsiaceae family that is native to the Mojave and Sonoran deserts of Mexico, California, and Arizona [SHAH *et al.,* 2010]. Jojoba seeds store lipids in the form of liquid wax that makes up 40–60% of their dry weight.

This wax has similar properties to sperm whale oil, and it is used as an industrial lubricant because of its superior lubricating ability and uniform viscosity over a wide range of temperatures [LLORENTE and APOSTOLO, 1998, BOSTAN *et al.,* 2013].

In addition, other uses of jojoba include diverse areas such as pharmaceutical, polishing, and gardening applications.

Jojoba is propagated by sexual and vegetative methods.

In plant populations derived by sexual propagation, it is difficult to determine sex type in early stages of growth i.e., 3–4 years from germination, and plants are genetically variable, which affects growth uniformity, physiological characteristics, yield and early bearing [MOHASSEB *et al.,* 2009, ROUSSOS *et al.,* 1999].

Further, jojoba is biased towards male (5:1; male: female ratio) [Al–OBAIDI *et al.,* 2012].

On the other hand, vegetative propagation methods provide genetically, uniform plant material with early fruiting.

Vegetative propagation can be achieved by rooting semi–hardwood cuttings but the maximum number of possible propagules is limited by plant size and time of year.

Thus, *in vitro* propagation offers opportunities for the production of thousands of elite plants from the selected stock plant.

Jojoba plants from tissue culture grow more vigorously than both seedlings and rooted cuttings, and are significantly larger after the first year of growth [BIRNBAUM *et al.,* 1985, BUTNARIU and BOSTAN, 2011].

Multiple shoots can be produced *in vitro* and these can be developed into plantlets by regenerating their roots.

Thus a single explant source, shoot tip or nodal segment could conceivably provide thousands of new true to type plantlets per year.

A number of workers have described *in vitro* culture of shoot tips [ELHAG *et al.,* 1998, SARDANA and BATRA 1998], nodal

segments [KACKER *et al.*, 1993, LLORENTE *et al.*, 1996, AGRAWAL *et al.*, 2002], polynodal segments/microcuttings [JACOBONI and STANDARDI 1987, JORDAN *et al.*, 1988,] for multiplication of jojoba clones.

Culture media containing different concentrations of various growth regulators have been used for *in vitro* shoot initiation of jojoba by a number of researchers.

Mostly cytokinins (BAP [*6-Benzyl amino purine*], Kinetin and Zeatin) have been used in combination with auxins (NAA [*2-(1-Naphthyl)acetic acid*], IAA [*indole-3-acetic acid*], and IBA [*Indole-3-butyric acid*]) or GA_3 [*Gibberellic Acid*] with varying levels of success.

Botti and Zunino used MS medium containing different concentrations and combinations of BAP, NAA and GA_3, the best response was obtained from 2 mg/L BAP in the medium [BOTTI and ZUNINO, 1988].

Micropropagated shoots cultured on MS/2 medium containing 3 mg/L IBA gave 31.08% rooting after 70 days [APOSTOLO *et al.*, 1996, RASHED and BUTNARIU, 2014a].

The objective of the present study was to propagate *Simmondsia chinensis* in commercial quantities via tissue culture technique, to produce true–to–type plants using direct organogenesis.

Material and methods

Collection of explants

One and half–year–old greenhouse jojoba plants (*S. chinensis*) propagated by seeds were used as the source of nodal segments with two full and half leaves and shoot tip explants for the present investigation.

Forty–day–old new shoots measuring 15–20 cm in length with four to six nodes were collected in polythene bags and brought to the laboratory for further treatments.

Preparation and sterilization of explants

Nodal segments with two full or half leaves and shoot tips 1.0–1.5 cm in length were excised under laminar air flow hood (LAF) and surface sterilized.

Explants were washed under running tap water for ten min (minute) and dipped in a solution of 500 mg/L Tetracycline (Antibiotic) and 4 g/L Yamastin ((Fungicide, Carbendaizm 60 WP) (Yamama Co., Ltd., Jordan) for 15 min followed by three rinses in distilled water. They were treated with 70% ethanol (Changshu Yangyuan Chemical, analytical reagent, China) for 30 sec (second) followed by three washes for one to two min with distilled water.

Under LAF, the shoot tips and nodal segments were immersed in 0.1% $HgCl_2$ (Mercuric chloride) [(w/v) (Qualigens, Mumbai, India)] for five and ten min, respectively.

After treatment both explants were again washed three times for one to two min with sterile distilled water and inoculated onto culture medium (Table 1).

Culture media and incubation conditions

The nutrients in growth media consisted of Murashige and Skoog [MURASHIGE and SKOOG, 1962, BUTNARIU *et al.*, 2014] (MS) basal salts additional to Gamborg's [GAMBORG'S, 1968, RASHED and BUTNARIU, 2014b] (B5) vitamins and modified MS medium (MMS) containing additional to the basal salts, the following compounds *viz.*, Nicotinic acid, Thiamin HCl, Pyridoxine HCl and Ca pentothenate all at 1.0 mg/L and the rooting medium was MS/2–B5 supplemented with IBA concentrations.

The medium was solidified with 7% agar bacteriological (Himedia, Mumbai, India), and supplemented with 3% sucrose (normal sugar available in the market, Alousra) except in rooting medium where the percentage of sucrose was 2 % and different types of PGRs.

The pH of the medium was adjusted to 5.8 after gelling with agar (bacteriological agar) with 1N NaOH or 1N HCl. The growth medium was then dispensed into 25–mL test tubes or onto 250–mL culture bottles and autoclaved for 15 and 20 min at 121°C respectively.

The cultures were incubated at 25±2°C under a 16–hrs photoperiod under cool white fluorescent light (37.5 µmol m^{-2} s^{-1}).

Shoot proliferation

The experiment consisted of 12 treatments i.e., three explants (nodal

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segments with two full or half leaves and shoot tips) and different PGRs i.e., BAP at 2.5 mg/L, GA_3 at 0.5 mg/L, IBA, NAA, and IAA all at 0.1 mg/L (Table 1, 2).

The explants were inoculated onto culture bottles with 40 mL or test tubes with 15 mL following surface sterilization.

The data of different shoot proliferation parameters *viz*., number of days to shoot initiation, number of shoots per explant, number of leaves and nodes per shoot, and shoot length were recorded 40 and 60 days after culture.

Shoot multiplication

The experiment consisted of six treatments i.e., three explants (nodal segments with two full or half leaves and shoot tips) derived from *in vitro* shoots and MS–B5 supplemented with 30 g sucrose and 1.0 mg/L BAP with or without 0.3 GA_3 mg/L (Table 3, 4).

The data of different parameters of shoot multiplication *viz*., number of regenerated shoots, number of leaves and nodes per shoot and shoot length were recorded 40 and 60 days after subculture.

Root induction

The experiment consisted of 3 treatments i.e., excised shoots with a minimum stem length of 2–3 cm were transferred to medium with MS/2– B5, and IBA at 1.0, 3.0, and 5.0 mg/L for the randomly selected population (Table 5).

The data of different parameters of rooting *viz*., percentage of rooting, number of roots per shoot and the root length (cm) were recorded for each shoot 60 days after culture additional to the number of days to root induction according to its emergence.

Experimental design and data analysis

Experiments were conducted in a factorial completely randomized design (FCRD) or completely randomized design (CRD) with three replicates, each with 10 explants per replicate.

Percentage data obtained for various parameters was square root transformed before analyzing data according to Gomez and Gomez, and Sastry [GOMEZ and GOMEZ, 1983; SASTRY 2007].

ANOVA values were obtained with Opstat1 software (O.P Sheron, Programmer, Computer Section, CCS HAU, Hisar, India) and means were separated with least significant difference (LSD) at P = 0.05.

Results and discussion

Shoot sterilization and proliferation

Almost all produced cultures from nodal segment with two full or half leaves and shoot tip explants treated with above procedure resulted in 85–100% uncontaminated culture (Table 1).

Contamination has often a major hurdle in establishing *in vitro* cultures with up to 90% losses [CHATURVEDI and SHARMA 1989].

In our work only less than 15 per cent was lost; this was due to using antibiotic (Tetracycline) with fungicide (Yamastin) and disinfectant chemicals (Ethanol + $HgCl_2$) additional to the source of explants from greenhouse.

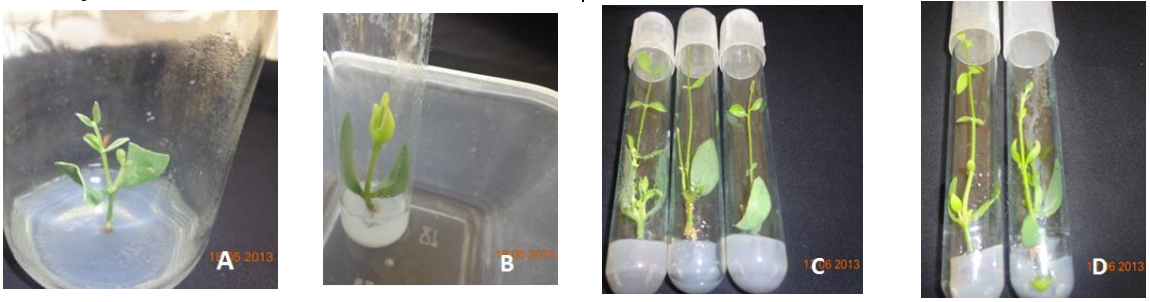

Figure 1. ***In vitro*** **shoot formation of jojoba**. Shoot formation 40 days after culture nodal segment explants (**A**) with two half leaves (**B**) with two full leaves in MS–B5 medium + 2.5 mg/L BAP + 0.5 mg/L GA $_3$ + 0.1 mg/L IBA; Shoot formation 60 days after culture nodal segment explants (**C**) with two half leaves (**D**) with shot tip explants in MS–B5 + 2.5 mg/L BAP + 0.5 mg/L GA $_3$ + 0.1 mg/L IBA.

This result is in line with our previous findings when produced Rangpur lime clean culture on *in vitro* by the use of fungicides, antibiotics and disinfectant chemicals such as Ethanol and $HgCl_2$ [EED *et al.*, 2010].

Proliferation of shoots for different parameters of jojoba was studied (Table 1; Figures 1A and 1B).

Table 1.

Effect of growth media and plant growth regulators on establishment of jojoba from nodal segment and shoot tip explants 40 days after culture

Plant growth regulators (mg/L)							Clean culture (%) (Mean)*	No. of days to shoot initiation (Mean)*	No. of shoots / explant (Mean)*	No. of leaves/ shoot (Mean)*	No. of nodes/ shoot (Mean)*	Shoot length (cm) (Mean)*
Media	Explant	BAP	GA_3	IBA	NAA	IAA						
MS–B5	N. segment[1]	2.5	0.5	0.1	–	–	100.00a (10.05)[3]	40.00 a	1.00 c	1.33 d	1.00 c	0.13 g
	N. segment[2]	2.5	0.5	0.1	–	–	100.00a (10.05)	40.00 a	1.00 c	1.33 d	1.00 c	0.13 g
	Shoot tip	2.5	0.5	0.1	–	–	91.66b (9.62)	10.33 b	2.33 a	9.33 a	2.33 ab	3.50 b
MS–B5	N. segment[1]	2.5	0.5	–	0.1	–	100.00a (10.05)	14.33 b	1.33 bc	2.00 d	1.66abc	2.00cdef
	N. segment[2]	2.5	0.5	–	0.1	–	100.00a (10.05)	14.33 b	1.00 c	4.00bcd	1.66abc	2.00cdef
	Shoot tip	2.5	0.5	–	0.1	–	85.00c (9.27)	14.00 b	1.00 c	2.00 d	1.00 c	1.16defg
MS–B5	N. segment[1]	2.5	0.5	–	–	0.1	100.00a (10.05)	11.66 b	1.33 bc	3.33bcd	1.00 c	0.73fg
	N. segment[2]	2.5	0.5	–	–	0.1	100.00a (10.05)	11.66 b	2.00 ab	4.66bcd	1.66abc	2.33bcde
	Shoot tip	2.5	0.5	–	–	0.1	95.00 b (9.79)	10.33 b	2.00 ab	3.33bcd	2.33 ab	3.33bc
MS–B5	N. segment[1]	2.5	0.5	–	–	0.1	100.00a (10.05)	11.66 b	1.33 bc	3.33bcd	1.00 c	0.73fg
	N. segment[2]	2.5	0.5	–	–	0.1	100.00a (10.05)	11.66 b	2.00 ab	4.66bcd	1.66abc	2.33bcde
	Shoot tip	2.5	0.5	–	–	0.1	95.00b (9.79)	10.33 b	2.00 ab	3.33bcd	2.33 ab	3.33bc
MMS	N. segment[1]	2.5	0.5	0.1	–	–	100.00a (10.05)	12.33 b	2.00 ab	6.00abc	2.66 a	2.66bcd
	N. segment[2]	2.5	0.5	0.1	–	–	100.00a (10.05)	12.33 b	2.00 ab	2.66 cd	1.33bc	1.10efg
	Shoot tip	2.5	0.5	0.1	–	–	95.00 b (9.79)	14.33 b	1.33 ab	6.66 ab	2.00abc	5.00 a

*Similar letters indicate means which are not significantly different (LSD, P = 0.05), comparisons are made in each column, values represent as means. [1]Nodalsegment with two full leaves [2]Nodal segment with tow half leaves[3] Data inside brackets are square root transformed values

Initiation of shoots after 40 days of inoculation of explants almost was 100 % (data not shown). This maximum response for shoot initiation was owing to applying of cytokinins *viz*., BAP with auxins *viz*., IBA, NAA and IAA. This might be attributed to cytokinins, which break bud dormancy by activating meristems and causing shoots to proliferate [MURASHIGE, 1974, BUTU *et al*., 2014].

The outgrowth of axillary buds is in general related with the cytokinin level in the buds. Initiation rates reported here were similar to those reported by Bashir and collab. and Roussos and collab. [BASHIR *et al.*, 2008; ROUSSOS *et al.*, 1999].

Early shoots formation was obtained in MS–B5 medium containing BAP at 2.5 mg/L, GA_3 at 0.5 mg/L and IBA at 0.1 mg/L after 10.33 days with shoot tip explants.

Similarly, same result of shoot formation was recorded in the previous medium with shoot tip explants but with substituting IBA with IAA at 0.1 mg/L.

The longest period for shoot formation (40.00 days) was noticed with nodal segments with two full and half leaves in the medium of MS–B5 supplemented with BAP at 2.5 mg/L, GA_3 at 0.5 mg/L and IBA at 0.1 mg/L.

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Almost all initiation media produced shoots early with both explants within 10.33–14.33 days with no significant difference except with the medium already mentioned above.

After 40 days of growth, higher number of shoots and leaves per explant was produced with shoot tip explants in MS–B5 medium supplemented with BAP at 2.5 mg/L, GA_3 at 0.5 mg/L and IBA at 0.1 mg/L compared to other explants and media, the values were 2.33 shoots and 9.33 leaves respectively.

The greatest number of nodes per shoot was achieved in the MMS medium with nodal segment with two full leaves compared to other explants and media.

Shoots were the longest (5 cm) significantly in the MMS medium comprising BAP at 2.5 mg/L, GA_3 at 0.5 mg/L and IBA at 0.1 mg/L with shoot tip explants in comparisons with other explants and media whereas the shortest shoots (0.13 cm) were obtained in MS–B5 medium containing BAP at 2.5 mg/L, GA_3 at 0.5 mg/L and IBA at 0.1 mg/L with the both type of explants.

However, after 60 days of growth, MS–B5 medium supplemented with BAP at 2.5 mg/L, GA_3 at 0.5 mg/L and IBA at 0.1 mg/L with shoot tip explants recorded significantly the greatest number of shoots per explant (2.33 shoots) and with same trend, MS–B5 medium containing BAP at 2.5 mg/L, GA_3 at 0.5 mg/L and NAA at 0.1 mg/L with nodal segment with two full leaves explants and MMS medium comprising BAP at 2.5 mg/L, GA_3 at 0.5 mg/L and IBA at 0.1 mg/L with nodal segment with two full leaves explants gave same result (2.33 shoots) (Table 2, Figures 1C and 1D).

Table 2.

Effect of growth media and plant growth regulators on establishment of jojoba from nodal segment and shoot tip explants 60 days after culture

Plant growth regulators (mg/L)							No. of shoots /explant (Mean)*	No. of leaves/ shoot (Mean)*	No. of nodes/ shoot (Mean)*	Shoot length (cm) (Mean)*
Media	Explant	BAP	GA_3	IBA	NAA	IAA				
MS–B5	N. segment[1]	2.5	0.5	0.1	–	–	1.00 b	1.66 f	1.00 c	0.23 c
	N. segment[2]	2.5	0.5	0.1	–	–	1.00 b	1.66 f	1.00 c	0.23 c
	Shoot tip	2.5	0.5	0.1	–	–	2.33 a	10.00 a	2.66 ab	5.00 b
MS–B5	N. segment[1]	2.5	0.5	–	0.1	–	2.33 a	2.66 ef	2.33 ab	4.33 b
	N. segment[2]	2.5	0.5	–	0.1	–	1.66 b	4.66 cde	2.00 bc	4.00 b
	Shoot tip	2.5	0.5	–	0.1	–	1.00 b	2.33 ef	1.00 c	1.50 c
MS–B5	N. segment[1]	2.5	0.5	–	–	0.1	1.33 b	3.66 def	1.00 c	1.73 c
	N. segment[2]	2.5	0.5	–	–	0.1	1.66 b	5.33 bcd	3.33 a	8.00 a
	Shoot tip	2.5	0.5	–	–	0.1	1.33 b	4.00 def	2.66 ab	4.33 b
MMS	N. segment[1]	2.5	0.5	0.1	–	–	2.33 a	6.66 bc	3.00 ab	4.33 b
	N. segment[2]	2.5	0.5	0.1	–	–	1.66 b	3.33 def	2.00 bc	4.00 b
	Shoot tip	2.5	0.5	0.1	–	–	1.33 b	7.33 b	3.33 a	8.00 a

*Similar letters indicate means which are not significantly different (LSD, P = 0.05), comparisons are made in each column, values represent as means. [1]Nodal segment with two full leaves [2]Nodal segment with tow half leaves

Similarly, MS–B5 medium supplemented with BAP at 2.5 mg/L, GA_3 at 0.5 mg/L and IBA at 0.1 mg/L with shoot tip explants obtained significantly the greatest number of leaves per shoot (10 leaves) compared to other explants and media.

The highest number of nodes per shoot and the longest shoots were observed with nodal segments with two half leaves in MS–B5 medium supplemented with BAP at 2.5 mg/L, GA_3 at 0.5 mg/L and IAA at 0.1 mg/L (3.33 nodes and 8.00 cm respectively) and also in MMS medium supplemented with BAP at 2.5 mg/L, GA_3 at 0.5 mg/L and IBA at 0.1 mg/L with shoot tip explants (3.33 nodes and 8.00 cm respectively).

From the mentioned results already, almost the shoot tip explants were more responsive for shoot proliferation in different parameters studied, perhaps due to presence of undeveloped buds.

According to Singh and Singh [SINGH and SINGH, 2005], cytokinins generally act in combination with auxins, cytokinins stimulate cell division even in non–meristematic tissues. In parenchyma, cell division occurs only when both auxins and cytokinins are present.

Furthermore, the ratio of cytokines to auxins controls cell differentiation and when the ratio is in the favour of cytokinins, shoot formation takes place.

Shoot multiplication

Table 3 and Figure 2A showed differences in the values among various explants and media after 40 days of multiplication but with no significant difference with respect to the characters of number of shoots per explant and number of leaves and nodes per shoot.

Table 3.

Effect of growth media and plant growth regulators on multiplication of *in vitro* jojoba nodal segment and shoot tip explants 40 days after culture

Media	Explant	Plant growth regulators (mg/L)		No. of shoots / explant (Mean)*	No. of leaves / shoot (Mean)*	No. of nodes / shoot (Mean)*	Shoot length (cm) (Mean)*
		BAP	GA_3				
MS–B5	N. segment[1]	1.0	0	2.00 a	5.66 a	3.66 a	3.66 c
	N. segment[2]	1.0	0	6.33 a	11.00 a	4.66 a	4.50 bc
	Shoot tip	1.0	0	3.66 a	8.33 a	3.66 a	5.00 ab
MS–B5	N. segment[1]	1.0	0.3	1.66 a	6.66 a	3.00 a	3.83 c
	N. segment[2]	1.0	0.3	4.66 a	10.00 a	3.66 a	5.83 a
	Shoot tip	1.0	0.3	2.66 a	6.66 a	3.66 a	3.66 c

Similar letters indicate means which are not significantly different (LSD, P = 0.05), comparisons are made in each column, values represent as means. [1]Nodalsegment with two full leaves. [2]Nodal segment with tow half leaves

The greatest number of shoots per explant and number of leaves and nodes per shoot (6.33–11.00–4.66 respectively) was recorded in MS–B5 with BAP at 1.0 mg/L and without GA_3 with nodal segments with two half leaves.

The highest shoot length was seen with nodal segment explants with the two half leaves in MS–B5 medium containing BAP at 1mg/L and GA_3 at 0.3 mg/L, the value was 5.83 cm.

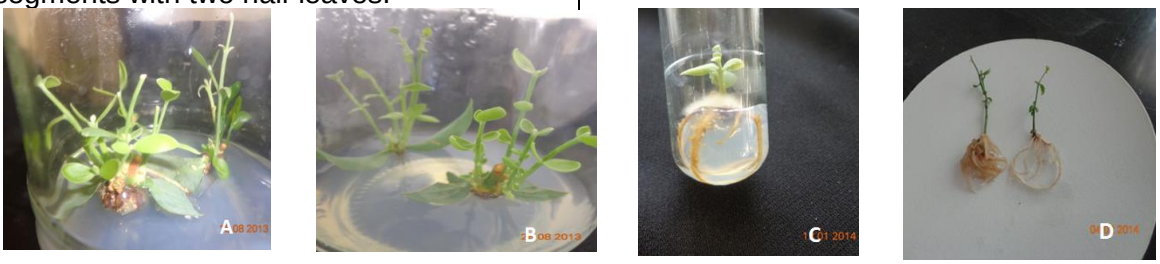

Figure 2. *In vitro* multiplication and rooting of jojoba. (**A**) Shoot multiplication 40 days after subculture *in vitro* nodal segment explants in MS–B5 medium + 1.0 mg/L BAP; (**B**) shoot multiplication 60 days after subculture *in vitro* nodal segment explants in MS–B5 medium + 1.0 mg/L BAP(**C–D**) Root induction 60 days after subculture shoots in MS/2–B5 + 1.0 mg/L IBA

While after 60 days of growth of explants in the multiplication medium, no significant difference was observed in terms of number of shoots per explant and number of leaves per shoot (Table 4 and Figure 2B).

Table 4.

Effect of growth media and plant growth regulators on multiplication of *in vitro* jojoba nodal segment and shoot tip explants 60 days after culture

Media	Explant	Plant growth regulators (mg/L)		No. of shoots /explant (Mean)*	No. of leaves/ shoot (Mean)*	No. of nodes/ shoot(Mean)*	Shoot length (cm) (Mean)*
		BAP	GA_3				
MS–B5	N. segment[1]	1.0	0	2.66 a	10.00 a	4.66 b	9.66 a
	N. segment[2]	1.0	0	6.33 a	32.00 a	3.00 c	7.00 b
	Shoot tip	1.0	0	3.33 a	18.00 a	4.00 bc	6.66 b
MS–B5	N. segment[1]	1.0	0.3	2.00 a	8.00 a	4.33 b	6.33 b
	N. segment[2]	1.0	0.3	5.00 a	20.33 a	8.00 a	9.33 a
	Shoot tip	1.0	0.3	3.33 a	13.66 a	2.66 d	4.00 c

Similar letters indicate means which are not significantly different (LSD, P = 0.05), comparisons are made in each column, values represent as means. [1]Nodalsegment with two full leaves. [2]Nodal segment with tow half leaves

The highest number of shoots per explant (6.33) and number of leaves per shoot (32.00) were observed in the MS–B5 containing 1.0 mg/L BAP and without GA_3 with nodal segment explants with two half leaves compared to other explants.

Significantly, greatest number of nodes per shoot (8.00) was formed in the MS–B5 containing BAP at 1.0 mg/L and GA_3 at 0.3 mg/L with nodal segment explants with two half leaves in comparison with other explants.

The longest shoots (9.66 cm) were achieved in the MS–B5 medium with 1.0 mg/L BAP and without GA_3 with nodal segment explants with two full leaves followed by the same medium containing BAP at 1.0 mg/L and GA_3 at 0.3 mg/L (9.33 cm) with nodal segment explants with two half leaves with no significant difference between both of them but they differed significantly with the remaining values of shoot length.

Root induction

Stem cuttings of these plants have been reported to be difficult to root *in vitro* as well as in soil [ARAGAO and HOGAN, 1976; CHATURVEDI and SHARMA, 1989; GARCIA BERENGUER, 1992; EED and BURGOYNE, 2014].

Root induction was done successfully in MS/2–B5 medium comprising different concentrations of IBA with selected *in vitro* shoots (Table 5 and Figures 2C, 2D).

Table 5.

Effect of different concentrations of IBA on *in vitro* rooting of jojoba shoots in MS/2–B5 medium

Medium	No. of days to root formation (Mean)*	% Rooting (Mean)*	No. of roots/ shoot (Mean)*	Root length (cm) (Mean)*
MS/2–B5 + 1mg/L IBA	50 a	33.33 a (5.84)1	6.66 a	4.33 a
MS/2–B5 + 3mg/L IBA	44 a	24.44 a (5.03)	3.66 b	2.66 b
MS/2–B5 + 5mg/L IBA	45 a	11.10 b (3.44)	2.33 b	1.66 b

* Similar letters indicate means which are not significantly different (LSD, P = 0.05), comparisons are made in each column, values represent as means. [1]Data inside brackets are square root transformed values.

Almost rooting of jojoba shoots were formed within 44–50 days with no significant difference among various periods. The minimum concentration of IBA (1.0 mg/L) gave significantly the highest rooting percentage (33.33 %) compared to the other IBA concentrations with less quantity of callus.

This was followed by IBA concentration of 3 mg/L with rooting percentage of 24.44 % then IBA at 5 mg/L with rooting percentage of 11.10 % which was the lowest per cent obtained.

IBA was used for *in vitro* rooting because it is the most common growth regulator used to stimulate in vivo rooting [LOW and HACKETT, 1981] and the optimum IBA concentration in our experiment was 1 mg/L.

This result also agreed with the findings reported by Llorent and Apostolo [LLORENT and APOSTOLO, 1998].

Conclusions

An efficient protocol was standardized for micropropagating of jojoba plants. The best growth medium for shoot sterilization and proliferation was MS-B5 medium supplemented with BAP at 2.5 mg/L, GA_3 at 0.5 mg/L and IBA at 0.1 mg/L with shoot tip explants.

The MS/2 medium containing 1 mg/L IBA was ideal for root induction.

Acknowledgements

The authors highly thank the Jojoba Naturals Corporation for their cooperation and providing plant materials. Special thanks for the managing director of Tissue Culture Lab., Sana'a, Yemen, the Eng. Abdallah Ba Asher (the *ex*. manager), Mr. Mohammed Almashraei (the current manager), and all workers at the lab. for their help in providing necessary facilities.

References

1. Agrawal, V.; Prakash, S.; Gupta, S.C. Effective protocol for *in vitro* shoot production through nodal explants of *Simmondsia chinensis*. *Biologia Plantarum*, **2002**, 4: 449–453.
2. Al–Obaidi, J.R.; Suliman, B.K.; Al–Ani, N.K. Molecular–based marker for sex

differentiation of jojoba *in vivo* and *in vitro* Iraqi cultivars using RAPD–PCR technique. *Scientific Research and Essays*, **2012**, 7: 522–527.

3. Apostolo, N.M.; Llorente, B.E.; Princen, L.H.; Rossi C. Rooting and acclimatization of migropropagated jojoba seedlings. *In:* Proc. of the 9th International Conference on Jojoba and Its Uses and of the 3rd International Conference on New Industrial Crops and Products, 25–30 September 1994, *Catamarca, Argentina*. **1996**, pp. 47–49.
4. Aragao, G.M.; Hogan, L.M., Crecimento e diferenciacao de tecidos de jojoba, *Simmondsia chinensis* (Link) Schneid, *in vitro*. *Ciencia agronomica*, **1976**, 6: 75–84.
5. Bashir, M.A.; Anjum M.A.; Rashid H. *In vitro* propagation of some promising genotypes of jojoba (*Simmondsia chinensis*). *African Journal of Biotechnology*. **2008**, 7: 3878–3886.
6. Birnbaum, E.; Matias, S.; Wenkart, S., Vegetative propagation of jojoba by tissue culture. *In:* Proc of the 6th International Conference on Jojoba and Its Uses, **1984**, *Beer–Sheva, Israel*. 233–241.
7. Bostan, C.; Butnariu, M.; Butu, M.; Ortan, A.; Butu, A.; Rodino, S.; Parvu, C. Allelopathic effect of *Festuca rubra* on perennial grasses. *Romanian Biotechnological Letters*, **2013**, 18(2): 8190–8196.
8. Botti, C.; Zunino, C. *In vitro* micropropagation of jojoba (*Simmondsia chinensis*). *Simiente*, **1988**, 58:11.
9. Butnariu, M.; Bostan, C. Antimicrobial and anti-inflammatory activities of the volatile oil compounds from *Tropaeolum majus* L. (Nasturtium), *African journal of biotechnology*, **2011**, 10(31): 5900–5909.
10. Butnariu, M.; Rodino, S.; Petrache, P.; Negoescu, C.; Butu, M. Determination and quantification of maize zeaxanthin stability, *Digest Journal of Nanomaterials and Biostructures*, **2014**, 9 (2), 745–755.
11. Butu, M.; Rodino, S.; Butu, A.; Butnariu M. Screening of bioflavonoid and antioxidant activity of *Lens culinaris* medikus. *Digest Journal of Nanomaterials & Biostructures*, **2014**, 9(2): 519–529.
12. Chaturvedi, H.C.; Sharma, M. *In vitro* production of cloned plants of jojoba (*Simmondsia chinensis* (Link) Schneider) through shoot proliferation in long term culture. *Plant Sciences*. **1989**, 63: 199–207.
13. Eed, A.M.; Amrender, Reddy, S.; Malla, Reddy, K.; Teixeira da Silva, J.A.; Veera Reddy, P.; Beghum, H.; Venkatasubbaiah P.Y. Effect of antibiotics and fungicides on the *in vitro* production of *Citrus limonia* Osbeck nodal segment and shoot tip explants. The Asian and Aust. *J. of Plant Sci. and Biotech*. **2010**, 4(1): 66–70.
14. Eed, A.M.; Burgoyne, A.H. Effect of different rooting media and plant growth regulators on rooting of jojoba (*Simmondsia chinensis* (Link) Schneider) semi–hard wood cuttings under plastic tunnel conditions. *In: Proc. of the International Conference on Agricultural, Ecological, and Medical Sciences*, Sandhu, S. Yingthawornsuk, T. (Eds.), 6–7 February **2014**, Bali, Indonesia. p. 14–17.
15. Elhag, H.; El–Olemy, M.M.; Mossa, J.S.; Tag–El–Din, S.S.; Al–Zoghet, M.F.; Al–Alsheikh, A.M.A. *In vitro* propagation of jojoba. *Program Abstracts of Annual Conference on New Crops and New Uses: Biodiversity and Sustainability*, 8–11 November **1998**, Phoenix, Arizona, USA.
16. Gamborg O.L.; Miller, R.; Ojima, K. Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research*. **1968**, 50:151–158.
17. Garcia Berenguer, A.; Gonzalez, D. Enraizamiento *in vitro* e *in vivo* de plantas seleccionadas de jojoba *Simmondsia chinensis* (Link) Schneider. *In: Memories of the Forth Latin American Conference on Jojoba*. La Rioja, Argentina. *Geeta Somani Agrotech Publishing Academy, Udaipur, India*. **1992**, 54–56.
18. Gomez, K.A.; Gomez, A.A. Statistical procedures for agricultural research. *John Wiley & Sons, New York*. **1983**, pp. 298–308.
19. Jacoboni, A.; Standardi, A. Tissue culture of jojoba (*Simmondsia chinensis*

Link). *Acta Horticulturae.* **1987**, 212: 557–560.
20. Jordan, M.; Arce, P.; Roverraro, C. Micropropagation *in vitro* and via cuttings in jojoba. *Ciencia–e–Inves. Agraria*, **1988**, 15: 31–37.
21. Kacker, N.L.; Joshi, S.P.; Singh, M.; Solanki, K.R. *In vitro* regeneration of female plants of *Simmondsia chinensis* (Link) Schneider (jojoba) using coppice shoots. *Annals of Arid Zone*. **1993**, 32: 175–177.
22. Llorente, B.E.; Apostolo, N.M. Effect of different growth regulators and genotype on *in vitro* propagation of jojoba. *New Zealand Journal of Crop and Horticultural Science*. **1998**, 26: 55–62.
23. Llorente, B.E.; Apostolo, N.M.; Princen, L.H. Rossi, C. Micropropagation of jojoba: effect of hormonal and clonal variation at the multiplication stage. *In: Proc. of the 9th International Conference on Jojoba and Its Uses and of the 3rd International Conference on New Industrial Crops and Products*, 25–30 September **1994**, Catamarca, Argentina. 50–52.
24. Low, C.B.; Hackett, W.P. Vegetative propagation of jojoba. *California Agriculture*. 35: 12–13.
25. Mohasseb, H.A.A.; El–Bahr, M.K.Z.; Adam, M.A.; Moursy, H.A.; Solliman, M.E. *In vitro* clonal propagation (*Simmondsia chinensis* (Link) Schn). *Australian Journal of Basic and Applied Sciences*. 1981, **2009**, 3: 3128–3136.
26. Murashige, T. Plant propagation through tissue cultures. *Annual Review of Plant Physiology*. **1974**, 25:135–166.
27. Murashige, T.; Skoog, F. A revised medium for rapid growth and bio–assay with tobacco tissue cultures. *Plant Physiology*. **1962**, 15: 473–497.
28. Rashed, K.; Butnariu, M. Antimicrobial and Antioxidant Activities of *Bauhinia racemosa* Lam. and Chemical Content, *Iranian journal of pharmaceutical research*, **2014**, 13 (3), 1073–1080b.
29. Rashed, K.; Butnariu, M. Isolation and Antimicrobial and Antioxidant Evaluation of Bio-Active Compounds from *Eriobotrya Japonica* Stems, *Advanced pharmaceutical bulletin*, **2014**, 4(1), 75–81a.
30. Roussos, P.A.; Toila–Marioli, A.; Pontikis, C.A.; Kotsias, D. Rapid multiplication of jojoba seedlings by *vitro* culture. *Plant Cell*, *Tissue and Organ Culture*. **1999**, 57: 133–137.
31. Sardana, J.; Batra, A. *In vitro* regeneration of jojoba (*Simmondsia chinensis*): a plant of high potential. *Advances in Plant Sciences*. **1998**, 1: 143–146.
32. Sastry, E.V.D. Essentials of Agricultural Statistics Pointer Publishers, Jaipur (India). **2007**, p. 260–266.
33. Shah, S.N.; Sharma, K.S.; Moser, B.R.; Erhan, S.Z. Preparation and evaluation of jojoba oil methyl esters as biodiesel and as a blend component in ultra–low sulfur diesel fuel. *BioEnergy Research*. **2010**, 3: 214–223.
34. Singh D.K.; Singh S.K. Physiology and post–harvest management of horticultural crops. **2005**.

Received: February 19, 2015
Article in Press: March 17, 2015
Accepted: Last modified on