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In vitro propagation of two jojoba elite clones cultivated in El- Magara

Reda Elsayed Abo El-Fadl
Tissue Culture Unit, Department of Plant Genetic Resources, Desert Research Center, El-Matarya11753, Cairo,Egypt

***Abstract**: Two elite jojoba clone varieties were used tostudy. Micropropagation techniques provide a faster and more economical solution to the limitations associated with traditional methods of plant cultivation. Experimentswere conducted for the optimization of shooting and rooting media. Micropropagation is an alternative technique to produce clonal plants in large-scale; the present study focuses on the multiple shoot and rooting induction. Stem nodal segments were cultured on MS medium supplemented with0.75 mg/l (6-benzyl-adenine (BA), and 0.1mg/l naphthalene acetic acid (NAA) was found to be suitable for shoot initiation. The effect of different types of cytokinin on the multiplication of shoots was investigated. MS medium containing 2.0mg/l BA in combination with 1.0 mg/l (kinetin) Kin and 0.1mg/l NAA gave the highest growth value. Additive casein hydrolysate (CH) and yeast extract (YE) for enhancing multiplication ate, at the concentration 75.0 mg/l CH improved shoot multiplication rate which gave 7.45 and 6.95 shoots per explant for bothclone1 (C1) and clone2(C2) varieties respectively. For rooting stage, half- strength MS medium containing 5.0 mg/l indole-butyric acid (IBA) in combination with0.2 mg/l NAA gave the highest rooting percentage with the highest mean number of roots and mean length of roots. Silver nitrate ($AgN0_3$) at different concentrations in half strength MSmedium and half strength Woody plant medium (WPM) containing 5.0 mg/l IBA was evaluated. The concentration of 2.0 mg/l $AgNO_3$ with half strength WPM medium gave the highest rooting percentage, highest mean number of roots and mean length of roots for both varieties. Finally, Rooted plantlets were acclimatized successively was achieved by using the media mixture of sand: peat moss (1:1: v: v) after six weeks.*

***Key words**: Jojoba, elites, micropropagation, stem nodal segments, additives*

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I. Introduction

jojoba (*Simmondsia chinensis*) (Link) Schn. is a nontraditional crop in arid and semi-arid areas. It is naturally well adapted to saline soils and high temperature environmental conditions. There is an increased interest in the agricultural production of jojoba and more promising experience has accumulated every year respecting cultivation requirements, planting densities, management practices, productivity, propagation techniques, and genetic improvement (Mill *et al*., 1997) and National Academy of Sciences (2002). Jojoba is a dioecious drought-tolerant perennial shrub (Harsh *et al*., 1987). Propagation by direct seeding has genetic heterogeneity and half of the seedlings are males. However, 8–10% males are necessary for pollination (Reddy and Chikara ,2010). The vegetative propagation of jojoba via conventional stem cuttings did not prove effective due to long procedure and slow growth (Lee *et al*. 1985).

Vegetative propagation was used to reproduce jojoba, e.g., layering (Reddy 2003), grafting (Bashir *et al*.2007), or semi- hard wood cuttings (Singh *et al*. 2003), but the number of propagules is limited by plant size and time of year. Micropropagation of elite individuals exploits totipotency of plant cells and offers a promising means of commercial mass production of pathogen-free superior clones. *In vitro*- derived jojoba plants grow more vigorously than both seedlings and rooted cuttings, and are significantly larger after the first year of growth (Chaturvedi and Sharma, 1989). Micropropagation is an alternative method of vegetative propagation, which is well suited to the multiplication of elite clones, offers many advantages, is not limited by number of selected elite genotype, produces pathogen-free plants, and can provide a commercial production within a limited time frame and space. The techniques can also be used for genetic improvement of the species (Reddy and Chikara, 2010). Jojoba is considered one of the most practical solutions for desert plantation in Egypt, heat, drought and salt tolerance, less possibilities for infection, less need for fertilizers and generous financial income, are certainly the most encouraging goals to plant jojoba in Egypt (El Moguy, 2002). Jojoba seed is rich in liquid wax, commonly mistaken for jojoba oil (Van Boven *et al*., 1997). The physical properties of jojoba oil are: high viscosity, high flash and fire point, high dielectric constant, high stability and low volatility. The cosmetic

industry appears to be the principal market for jojoba oil products, and the other major industry using jojoba oil is the pharmaceutical sector. Jojoba is dioecious; the female plants produce seed from flowers pollinated by the male plants. Female plants are commercially more important for the seed production (Harsh *et al*., 1987). Jojoba is considered a promising oil crop and is cultivated for diverse purposes in many countries. The jojoba seed produces unique high-quality oil with a wide range of applications such as medical and industrial-related products. The plant also has potential value in combatting desertification and land degradation in dry and semi-dry areas. Although the plant is known for its high-temperature and high-salinity tolerance growth ability, issues such as its male-biased ratio, relatively late flowering and seed production time hamper the cultivation of this plant. (Jameel *et al*.,2017). Jojoba oil and its derivatives were used widely in the industryfor producing pharmaceutical and medicinal products in addition to cosmetics such as (face creams, lipsticks, skin fresheners soaps and shampoos), antifoaming agents and resistant lubricants to high temperature and pressure (Reddy andChikara, 2010). synthetic polymer substitute (Ifuku,2017), and is used in the bioenergy industry (Le Dréau*et al*.,2009). Interestingly, jojoba oil has some medicinal properties such as the relief of headaches and throat inflammation and in treating wounds (Ranzato*et al*., 2011). Jojoba oil is reported to have anti-inflammatory activity, as well as antimicrobial (Habashy *et al*., 2005) and antifungal/insecticidal properties (Abdel-Mageed*et al*., 2016).

II. Material and Methods

1. Explant sources and sterilization

Jojoba explants were collected from two elite clone varieties, mature plants grown in Magara station, Desert Research Center, Sinai. Nodal stem segments were collected, moistened and wrapped in moistened newspaper, labeled, packaged into ice box container. Leaves were removed from the lowest node. They were washed with soap under running tap water with for 1 hour to remove all the remaining detergent. Surface sterilization was carried out in a transfer hood by soaking them in 50% (v/v) Clorox bleach solution (2.5% sodium hypochlorite) for 40 min, providing gentile agitation, followed by three sequential rinses in sterilized distilled water, then in 0.1 % (w/v) sterile solution of mercuric chloride for 3 minutes. All explants were rinsed for six times with sterile distilled water to remove the traces of $HgCl_2$ solution

2. Culture media and condition

Culture media consisted of full - strength MS (Murashige and Skoog, 1962) supplemented with 3% w/v sucrose, 100 mg/l myo-inositol and). The pH of the medium was adjusted to 5.7 -5.8 before being solidified with2.8 g/l phytagel (Duchefa, Haarlem, the Netherlands). Different concentrations of plant growth regulators and growth additives were added to the nutrient medium, according to the growth stage requirements. Media were dispensed in large jars capped with autoclavable polypropylene lids, then autoclaved at a pressure of 1.1 kg/cm_2 and $121c^o$ for 20 min. The surface sterilized explants were inoculated on the above media under aseptic conditions. The cultures were incubated at approximately 25+.co with a 16 – h photoperiod under cool white florescent tubes (Toshiba). Six-week-old, shoot initiation%, no. of shoots and mean length of shoots(cm) were recorded.

3. Shoot initiation

Nodal stem segments were cultured on MS medium, for shoot initiation, supplemented with 6- benzyl adenine (BA), at concentrations of 0.0, 0.25, 0.5, 0.75 and 1.0 mg/l in combination with 0.1 mg/l naphthalene acetic acid (NAA), in addition to PGRs- free MS medium as control treatment. After six weeks of culturing, shoot initiation %, mean number of shoots per explant and mean length of shoot were recorded.

4. Shoot multiplication

Primary shoots formed *in vitro* were cultured on MS medium containing BA, kinetin(kin) at concentrations of 1.0, 2.0 and 3.0mg/l individually or in combination with indole acetic acid (IAA) at concentration 0.1mg/l, in addition to the control medium (PGRs -free). No. of shoots per explant and mean shoot length were recorded after eight weeks.

5. Shoots multiplication with different additives

To increase shoot multiplication rate, casein hydrolysate (CH) and Yeast extract (YE) individually at concentrations 25.0, 50.0, 75.0 and 100.0 mg/l for each additive, with gibberellic acid (GA_3) at 0.5mg/l were added to the best multiplication media. No. of shoots and mean shoot length (cm). were recorded after eight weeks.

6.*In vitro* rooting

For rooting induction, shoots were transferred into half strength MS medium containing indole butyric acid (IBA) or indole acetic acid (IAA) (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) in combination with 0.2 mg/l of naphthaleneacetic acid (NAA) (Duchefa, Haarlem, the Netherlands). PGRs -free half MS medium was served as control. Rooting percentage %, mean root number and mean length of roots (cm) were recorded after eight weeks.

An experiment for rooting improvement was carried out using half strength MS and half strength WPM(Lloyd and McCown,1981) media using silver nitrate ($AgNO_3$), at different concentrations (0.5, 1.0, 2.0 and 3.0mg/l) on 5.0mg/l IBA. Rooting percentage %, no. of roots and mean length of roots were recorded after eight weeks.

7. Acclimatization

Plantlets with well-developed roots were removed from the culture medium and after washing the roots gently under running tap water and soaked in 2g/l fungicide solution (benlate). plantlets were transferred to plastic pots (13 cm diameter), containing mixture of sand and peat moss at equal volumes. Plantlets were covered with plastic bags and transferred to greenhouse. After four weeks, plastic bags were gradually removed from pots for proper hardening. After 4 weeks, acclimatized plants were transferred to pots containing normal soil and maintained in greenhouse under normal conditions and after one month, surviving plants were transferred to natural conditions.

8. Experimental design and statistical analysis of data

The experiments were subjected to a completely randomized design. Analysis of variance (ANOVA) and Duncan' s multiple range test (Duncan,1955), as modified by Snedecor and Cochron (1998) were used to analyze the obtained data. Each treatment had three replicated and each replicate consisted of 9 jars. The experiments were repeated twice. The differences between the averages of the recorded parameters for all treatments were tested for significance at the 5% level. The averages followed by the same letter are not significantly different at $p<0.05$.

III. Results and Discussion

1. Initiation stage

A suitable method *in vitro* propagation is thus needed to develop this plant as a crop, and also to multiply and maintain elite clones with desirable characteristics, wax content, seed yield and disease resistance. Micropropagation of jojoba plant offers a promising means of commercialmass production of superior clones. A single explant source could conceivably provide housands of true-to-type plantlets per year i.e., those plants that are genetically similar to the parental stock and that could potentially maintain the genetic line (Bekheet*et al*., 2015). The effect of BA and NAA on shoot initiation of jojoba was tested (Table 1). It was observed that, the effect of different concentrations of BA on the shoot initiation for the nodal segments of two clones (C1 and C2) Shoots initiation percentage was responded differently to the used treatments according to clones. In this concern, the combined growth regulators treatment 0.75 mg/l BA and 0.1 mg/l NAA proved to be the most effective on shoot initiation percentage, no. of shoots per explant and mean length of shoots (90%), 2.7 shoots and 3.5cm respectively, while the lowest significant value of shoot percentage, no. of shoots and mean length of shoots 44%, 1.2 shoots and 1.4 cm respectively for C1 was recorded for PGRs free medium(control). With regard the effect of clones, the obtained results showed that the highest significant value of shoot initiation percentage (90%) was for C1followed by C2 (85%). The results show that BA with NAA had positive effect on this parameter (Figure 2). Maximum numbers of shoots were regenerated on medium containing low concentration of 0.75 mg/l BA (Sharma *et al*., 2017). BA, had a significant effect on proliferation rate, shoot number, and length (Souhayla, *et al*.,2021). One of the most important groups of PGRs are the cytokinin (CKs) which play a crucial role in regeneration and shoots proliferation (Gaba,2005). Of the five cytokinins tested, BA at the three different concentrations was most effective in inducing bud break (Omaima *et al*., 2018).

Table 1: Effect of PGRs (BA and NAA) in MS medium on shoot initiation of two elite clone varieties of jojoba *in vitro* after six weeks.

Treatments(mg/l)		Jojoba clonevarieties					
		Clonal 1			Clonal 2		
BA	NAA	Shoot initiation %	no. of shoots	Mean length of shoots(cm)	Shoot initiation %	no. of shoots	Mean length of shoots(cm)
0.00	0.0	44 e	1.26 c	1.4 c	44 e	1.16 b	1.3 b
0.25	0.1	64 d	1.26 c	1.6 c	55 d	1.3 b	1.5b
0.50	0.1	75 c	2.33 b	2.1 b	70 c	2.13 a	1.9 b
0.75	0.1	90 a	2.76 a	3.5a	85 a	1.96 a	2.6a
1.00	0.1	88 b	1.56 c	1.7 c	75 b	1.4 b	1.5 b

Mean followed by the same letter within a column are not significantly different at $p<0.05$

The most useful and universally used cytokinin for *in vitro* culture is BA as it can metabolize immediately in plant tissues (Krikorian, 1995), and it was therefore used for shoots proliferation and shoot elongation. Therefore, selection of proper concentration of plant growth regulator is critical to shoot regeneration. An optimal concentration of cytokinin results in marked increase not only in RNA but also in DNA and protein synthesis leading to initiation of shoot primordia (Mok and Mok, 2001). concentrations of BA alone or in combination with NAA, BA at 0.5 and 1.0 mg/l were most suitable for shoot induction and longer shoots (Taye *et al*., 2018). Irrespective of concentrations and combinations of BA with other plant growth regulators the apical and axillary buds sprouted and elongated to 1.5 - 2.5 cm (Mallikarjuna and Rajendrudu,2007). The percentage of nodal explants sprouting was found to be increased with increasing the concentration of cytokinins. Of the three cytokinin tested, BA was found to be most effective (Mohd and Mohammad,2009). the exogenous BA and NAA had key regulation on the growth and contents of medicinal ingredient of *hellodendronchinense* seedlings (Hanjie *et al*., 2018). NAA and BA combinations however, have also been reported to be efficient in some plant species in the Asclepiadaceae family (Sudha *et al*.,1998 and Vinothkumar *et al*., 2011). However, it was significantly higher than mean shoot lengths recorded in the low BA concentrations (0.5 and 1.0 mg/l BA) (Maame *et al*., 2016). Efficacy of BA over kinetin for nodal bud break may be attributed to its easy metabolism by plant tissues than other cytokinins (Rai *et al*., 2010), easy permeability, less resistance for cytokinin oxidase and natural hormone induction within the tissue (Lodha *et al*., 2015). Similar results have also been observed in *Pithocellobium dulce* (Goyal *et al*. ,2012), *Moringa peregrine*(Al Khateeb *et al*., 2013), *Dalbergia sisso* (Vibha *et al*., 2014).

2. Multiplication stage

Data illustrated in Table (2) and Figure (3) exhibited the effect of tested MS medium supplemented with various levels of BA, Kin and NAA and their combinations on mean number of shoots per explant and mean length shoot length formed during multiplication stage of two jojoba clones. the results revealed that the number of shoots produced was significantly affected by the different concentration and type of cytokinin used. Regarding the mean number of shoots formed, the mean effect of BA and Kin, showed a very highly effect on the defined trait, especially when MS medium was supplemented with 2.0 mg/l BA in combination with 1.0 mg/l Kin, which recorded the highest mean as value (3.13) of shoots / explant, as compared to other concentrations. These results agree with results obtained by (Marwa *et al*.,2022). The interaction between BA, Kin concentration and Paulownia species clear that the number of shoots/explants reached its maximum value as P. hybrid and the interaction between BA, Kin concentration and Paulownia species (*P. hybrid* and *P. tomentosa*) clear that, both Paulownia species shoot length reached maximum values. Superiority of BA and kinetin in combination has been found for micropropagation of other woody perennials (Das *et al*., 1996; Komalavalli and Rao, 1997). The addition of BA, Kin and IAA induced a maximum shoots per explant (Kalimuthu *et al*.,2007). The earlier studies report that several types of explants of J curcas responded very positively to these hormones with respect to induction of callus, shoots, roots, somatic embryos (Sujatha and Mukta 1996, Jyoti-Sardana *et al*. 2000). On the other hand, the lowest mean number of shoots was obtained in 1.0 mg/l Kin and 0.1mg/l NAA. All combination of PGRs in this study produced multiple shoots, with the multiplication rate ranging from 1.63 to 3.13. Control medium without PGRs did not show any multiplication for growth of single shoot. The addition of auxins to the optimal concentration of Kin or BA significantly increased the frequency of shoot differentiation when compared to Kin or BA alone in the present study. At high

concentration of BA (3.0 mg/l) the shoots favored callus formation, and hyperhydricity. For the mean length of shoots, the main effect of Kin exerted a very highly significant effect on the given characteristic. For instance, supplementing MS medium with 1.0 mg/l Kin and 0.1 mg/l IAA gave the highest mean length (5.6cm) compare to other treatments. Concerning the effect of variety genotype, data demonstrate that, the highest mean number of shoots and mean length of shoots was attained with variety C1. It can be concluded that, the interaction between both growth regulators BA and Kin, exerted very highly significant effects on the multiplication rate, especially when both was added to MS culture medium at 2.0 mg/l and 1.0 mg/l, in series which led to the highest mean value.

Table 2: Effect of PGRs (BA, Kin and IAA) in MS medium on shoot multiplication after eight weeks.

Plant growth regulators (mg/l)			Jojoba clonevarieties			
			C_1		C_2	
BA	**Kin**	**IAA**	**no. of shoots per explant**	**Shoot length**	**no. of shoots per explants**	**Shoot length(cm)**
0.0	0.0	0.0	0.00 e	0.0 e	0.0 e	0.0 d
1.0	0.0	0.1	1.63 c	2.3 d	1.66 cd	2.4 c
2.0	0.0	0.1	1.96 c	3.0 c	1.9 c	2.9 c
3.0	0.0	0.1	2.00 c	2.4 d	1.83 cd	2.4 c
0.0	1.0	0.1	1.0 0d	3.2 c	1.46 d	2.9 c
0.0	2.0	0.1	1.90 c	4.1 b	1.83 cd	3.9 b
0.0	3.0	0.1	2.06 c	5.6 a	1.93 c	5.0 a
1.0	1.0	0.1	2.63 b	3.9 b	2.36 b	3.8 b
2.0	1.0	0.1	3.13 a	3.8 b	2.86 a	3.7 b

Mean followed by the same letter within a column are not significantly different at$p<0.05$

3. Shoots multiplication in MS media supplementedwith different additives and GA_3

Two elicitors, CH and YE were added separately, significantly, affected the shoot multiplication rate after two subcultures, when added to the MS medium containing 2.0 mg/l BA with 1.0 mg/l Kin and 0.1 mg/l IAA (the most suitable cytokinin level for shoot multiplication). Table (3). Among various concentration of additives in addition to the suitable MS medium for shoot multiplication. The inclusion of CH and YE, individually improve shoot multiplication rate in both variety C1 and C2 when added to MS medium. The highest mean shoots (7.45) per explant and mean length of shoots (8.3cm) were observed on MS medium supplemented with 75mg/l CH with variety C1. influenced shoot multiplication significantly. Supplied individually, CH improved shoot multiplication in variety FHIA-21(Oumar *et al*., 2017), capable of sustainable multiplication of shoots, was obtained for two banana varieties. Inclusion of CH is most effective for shoot multiplication (Priyanka *et al*., 2012)., plants treated with casein hydrolysate displayed the maximum regeneration rate in R. c*anina*. Inclusion of casein hydrolysate in proliferation media increasingly enhanced shoot regeneration in this species (Leila *et al*., 2021). Casein hydrolysate as a simple protein decomposes into glucosamine and galactosamine or mannosamine as well as simple sugars linked with proteins by covalent bonds or by glycoside bond with hydroxyl group of the amino acids serine and threonine (Al-Dalaly and Al-Rekaby,1995). While YE (100.0mg/l) gave the highest men shoots (6.59) per shoots and mean length of shoots (7.7cm) in variety C2. The higher concentration of both additives CH and YE (100.0 mg/l) was found inhibitory for shoot proliferation and elongation. YE increase shoot multiplication significantly when compared to the basal medium alone (Diako *et al*., 2021). These substances are generally utilized as potential additives besides plant growth regulators during various stages of *in vitro* plant propagation for the purpose of improving the plant quality and regeneration rate and thereby enhance the efficacy of this process (Sridhar and Aswath, 2014). Yeast extract is one such organic nutrient supplement, having all the essential nutrient, amino acid, and organic acids (George *et al*., 2008).

Table 3: Effect of casein hydrolysate (CH), Yeast extract (YE) and gibberellic acids (GA_3) supplemented MS medium containing 2.0 mg/l BA in combination with 1.0 mg/l Kin and 0.1mg/l IAA on shoot multiplication after eight weeks of culture

Treatments (mg/l)			Jojoba clonevarieties			
			C_1		C_2	
CH	YE	GA_3	no. of shoots per explant	Mean shoot length (cm)	no. of shoots per explant	Mean shoot length (cm)
25.0	0.0	0.5	4.82 d	5.0 b	4.4 cd	5.2 x
50.0	0.0	0.5	6.76 b	7.5 a	5.29 bc	6.4 a
75.0	0.0	0.5	7.45 a	8.3 a	6.20 ab	7.4 a
100.0	0.0	0.5	6.12 c	7.5 a	6.16 ab	7.6 a
0.0	25.0	0.5	3.5 e	5.1 b	3.7 d	5.0 b
0.0	50.0	0.5	4.72 d	7.2 a	4.52 cd	6.6 a
0.0	75.0	0.5	6.35 bc	7.2 a	5.75 ab	7.1 a
0.0	100.0	0.5	6.81 b	7.5 a	6.59 a	7.7 a

Mean followed by the same letter within a column are not significantly different at $p > 0.05$

4. Rooting

Formation of root is an important index of *in vitro* plantlets, quality and a critical factor for transplanting adventitious rooting. Three auxins tested for rooting of jojoba variety, presented in Table 4. All the treatments resulted in root production with frequencies ranging from 27 to 60%. The main effect of IBA showed that that IBA exerted very highly significant effect on the given trait. The highest rooting percentage of the shoots rooted in half- strength MS medium containing 5.0 mg/l IBA. This medium was the best rooting medium. It gave the highest mean number for both varieties C1 and C2, which gave 2.3 and 3.04 roots /shoot and length 4.4 and 4.9cm receptively (Table4). in agreement with previous study, Gopitha *et al*. 2010, reported that different varieties were found to response differently in producing roots. Similar results were obtained who attained highest rooting of shoots on MS medium supplemented with IBA (Safarnejad*et al*.,2011). Rooting of jojoba *in vitro* plantlets seemed to be difficult due to the low percentage of rooting obtained (Fayek *et al*., 2007). The higher concentration of 5mg/l IBA was found better for root regeneration in all the variety (Rahman *et al*.,2018). Dorić *et al*. (2014) who obtained the maximum number of roots and roots length in 'SV1' selection (*P. fruticosa*) at the highest IBA concentration. Root initial cells division depends on both Endogenous and exogenous auxins concentration. The physiological effects of auxins are represented in increasing of cell division or converting the material of differentiated cells in shoots bases into meristematic cells (totipotent cells) so adventitious root meristem will be formed and its cells will be divided to produce adventitious roots,Bdul, (1987). Saleh, (1991). Endogenous hormones might have a role in promoting shoots to root (peak *et al*.,1987) until the hormonal balance reached its optimal level to push the roots to grow and develop in presence of exogenous hormones, since increasing of auxin concentration promotes root formation on shoot bases, George and Shermington (1984). Roots can be regenerated from *in vitro* regenerated shoots on half MS medium supplemented with NAA, IBA and IAA (Khan *et al*., 2008 and Baksha*et al*., 2000). Rooting was highly influenced by the different types and concentrations of auxin used. Khan *et al*.,2009, and Singh*et al*.,2003, reported on the IBA and IAA, NAA was the most efficient auxin for root initiation of sugarcane *in vitro* propagation. (Jagadeesh *et al*., 2011).

Table 4: Effect of different concentrations of Indole butyric acid (IBA), indole acetic acid (IAA) and naphthalene acetic acid (NAA) on root formation of two elite clone varieties after six weeks

Treatments (mg/l)			Jojoba clone varieties					
			C_1			C_2		
IBA	IAA	NAA	Rooting %	no. of roots	Mean length of roots (cm)	Rooting %	no. of roots	Mean length of roots (cm)
0.0	0.0	0.0	0 l	0.0 f	0.0 g	0 k	0.0 h	0.0 f
0.5	0.0	0.2	27 j	0.54 e	1.7 f	33 i	0.55 g	1.9 e
1.0	0.0	0.2	37 h	0.89 d	1.8 f	40 h	1.0 ef	2.1 de
2.0	0.0	0.2	46 f	1.02 d	2.3 de	46 f	1.12 e	2.5 cde
3.0	0.0	0.2	50 d	1.85 b	2.5 d	53 d	2.31 c	2.7 cd
4.0	0.0	0.2	53 c	2.24 a	3.0 c	58 b	2.62 b	3.0 c
5.0	0.0	0.2	60 a	2.34 a	3.2 c	63 a	3.04 a	3.8 b
0.0	0.0	0.2	25 k	2.34 a	1.9 ef	29 j	0.55 g	2.0 de
0.0	0.5	0.2	32 i	0.17 f	2.2 def	34 i	0.84 f	2.6 cde
0.0	1.0	0.2	36 h	0.78 d	3.1 c	42 g	1.05cef	3.7 b
0.0	2.0	0.2	43 g	1.0 d	3.7 b	48 e	1.82 d	3.9 b
0.0	3.0	0.2	48 e	1.66 c	3.8 b	52 d	2.64 b	4.7 a
0.0	4.0	0.2	55 b	2.22 a	4.3 a	55 c	2.96 a	4.9 a

Mean followed by the same letter within a column are not significantly different atp<0.05

5. Effect of different media and $AgNo_3$ on rooting development

$AgNo_3$ combined with IBA with different media increased percent rooting, mean number of roots and mean length of roots (Table 5) and (Figure 5). It was observed that addition of $AgNo_3$ in the rooting induction medium at concentrations ranging from to 0.5 to 3.0 mg/l was successful in promoting rooting percentage from 60 to 88% and enhanced the mean number and length of roots, compared with control medium. The highest value in rooting percentage, number of roots and length were achieved with 2.0 mg/l $AgNo_3$ which gave 88%, 7.1 and 7.4 respectively, it could be noticed that parameters of roots were gradually increased with increasing the concentration from 0.5 to 2.0mg/l. Moreover, parameters were decreased with the higher concentration of $AgNo_3$. Concerning the effect type of media, it was It was found that half WPM was more effective than half MS medium for rooting formation which gave better result. The greatest percentage of root regeneration (88%) was obtained when shoots were placed on half-strength WPM with IBA and the maximum mean number of roots (7.2) and mean length of roots 7.7cm was regenerated for variety C2. while for MS medium supplemented with IBA the highest percentage of rooting (85%) was obtained for C2 genotype and the highest mean number of roots (6.1) and mean length of roots (6.4cm). The silver nitrate improved *in vitro* root formation of several species: *Decalepishamiltonii* (Bais *et al*., 2000 and REDDY *et al*., 2003. $AgNO_3$ has been employed in tissue culture studies for inhibiting ethylene action because of its water solubility and lack of phytotoxicity at effective concentrations (Beyer, 1976). The highest number of roots per shoot (Ankita *et al*., 2021). Plantlets were rooted on WPM medium with different concentrations of indole-3-butyric acid (IBA). Highest rooting percentage and survival was achieved on WPM medium (Singh *et al*., 2016). WPM medium containing IBA was most effective for rooting in comparison to MS medium. Rooted plantlets were successfully hardened and established in pots (Maliheh *et al*., 2015).

Table 5: Effect of different half strength media containing 5.0 mg/l IBA and different concentrations of $AgNo_3$ on rooting improvement of jojoba after Six weeks

Media	$AgNo_3$	Jojoba clonevarieties					
		C1			C2		
		Rooting%	no. of rooting	Mean length of roots(cm)	Rooting %	no. of roots	Mean length of roots(cm)
MS	0.0	60 e	2.27 f	1.7 f	63 a	3.04 d	3.8 e
	0.5	60 e	2.95 e	1.7 f	63 a	3.43 d	41. e
	1.0	66 d	5.14 c	2.7 de	68 a	4.95 c	5.4 cd
	2.0	77 b	5.78 b	4.1 ab	85 a	6.12 b	6.4 b
	3.0	70 c	4.98 c	3.3 cd	76 a	5.19 c	5.8 bcd
WPM	0.0	65 d	2.45 f	2.3 ef	71 a	3.52 d	3.8 e
	0.5	65 d	3.78 d	2.9 cde	71 a	3.67 d	4.4 e
	1.0	70 c	4.94 c	3.5 bc	76 a	5.38 c	5.2 d
	2.0	78 a	6.59 a	4.5 a	88 a	7.14 a	7.7 a
	3.0	75 b	548 b	4.1 ab	57 .33 a	5.99 b	6.2bc

Mean followed by the same letter within a column are not significantly different atp<0.05

6. Acclimatization

The rooted plantlets were carefully transferred from culture vessels into greenhouse, then washed gently with water to remove media traces to discourage infection by fungal contamination, and then plantlets into pots contain a mixture of sand and peatmoss (1: 1: v: v)and covered with a transparent bag to ensure high humidity for six weeks and the plastic bagswere gradually removed after four weeks to acclimatize under greenhouse conditions. A percentage 80 % for both two varieties. The *in vitro* raised plantlets have been transferred outside the greenhouse (Figure 6).

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Figure1: Fruits of jojoba from elite clones

Figure 2: Shoot initiation of two elite clones jojoba. Stem nodal segment cultures on MS medium containing 0.75 mg/l BA and 0.1 mg/l NAA

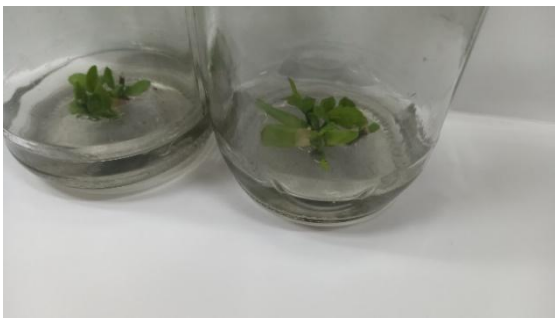

Figure 3: Shoot multiplication on MS medium containing 2.0mg/l BA and 1.0 ng/l Kin and 0.1mg/l IAA after four weeks.

Figure 4: Improvment Shoot multiplication on MSmedium contaning 2.0mg/l BA and 1.0 mg/l Kin and 0.1 mg/l IAA in addition 75.0 mg/l CH after eight weeks.

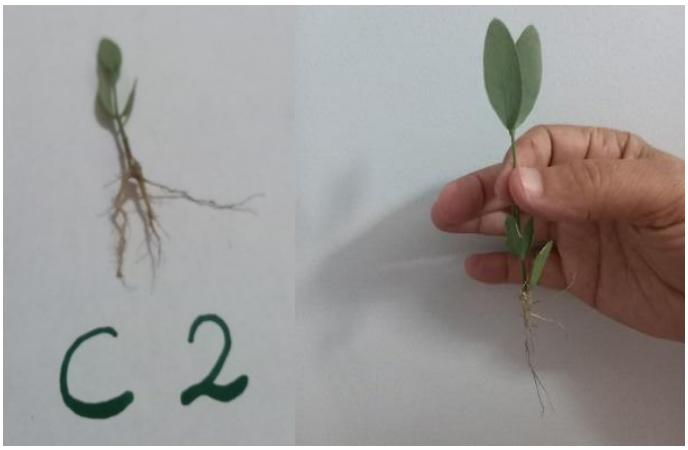

Figure 5: rooted plantlets of jojoba in half strength WPM medium containing 5.0mg/l IBA and 0.5 mg/l GA_3in addition to 2.0 mg/l $AgNo_3$

Figure 6: Acclimatized plantlets of two clones, a three -month- old acclimatized plantlets outside greenhouse.

Reda Elsayed Abo El-Fadl. *"In vitro* propagation of two jojoba elite clones cultivated in El-Magara.*"IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 15(06), 2022, pp. 62-72.