

Comparative studies on vegetative and in vitro propagation of elite selected jojoba strains

Abstract

The responses of five jojoba genotypes to *in vitro* nodal segments and shoot tip culture under different growth regulators combinations and sucrose concentrations were investigated. Explants taking time has a great effect on jojoba plant proliferation, it was noticed that the best time for taking explants begin from the mid of March to mid of April; moreover, type of tested explants revealed different response towards proliferation. Concentrations of different growth regulators played an important role on growth and proliferation of jojoba genotypes. It was clear that combination of BA+NAA (3 mgL⁻¹ and 0.5 mg L⁻¹ respectively) showed highest rate of multiplication in respect to shoot number, shoot length and leaves number. Regarding rooting, proliferated plants failed to initiate roots when different types of hormones (BAP, TDZ and NAA) with different concentration (0.5, 1 and 2 mg/L) were tested. However, the tested genotypes failed to initiate any roots. On the other hand, propagation via stem cutting treated with IBA at 3000 ppm concentration was also investigated. It was clear that by increase the rooting percentage the survival percentage increase.

Introduction

Jojoba (*Simmondsia chinensis*) is a perennial evergreen, dioecious and an obligated cross pollinated shrub. Jojoba is cultivated in arid and semi-arid regions. The Jojoba seeds contain about 50-55% distinctive oil, which was used commercially in the cosmetic, pharmaceutical and lubricant industries. Jojoba is considered a new industrial crop compatible to the nature of Egypt desert and there is a real need to expand the cultivation area without exceeding water quota and still generate profitable economic returns.

Several asexual methods of propagation have been used to propagate jojoba; each of these shares the major advantage over seed propagation in that they allow propagation of unique and desirable genotypes. An additional advantage of asexually propagated plants over several seedlings is that they have shorter juvenile period. Stem cuttings one of the asexual methods of propagation (Reddy *et al.*, 1980; Palzkill, 1988; Ayerza *et al.*, 1996; Basher *et al.*, 2001 and Hamad 2010).

Moreover, asexual propagation via tissue culture technique suited to the multiplication of elite crops by using explants of the plant leaf, root and stem. On the other hand *in vitro* culture techniques reproduce true to type plants and asexual propagation of single jojoba plant is necessary to conserve the desirable traits.

The objective of this study is to propagate five elite selected jojoba strains via cuttings and *in vitro* to demonstrate the most appropriate method for vegetative multiplication.

Materials and Methods

Plant materials

Five selected elite genotypes of jojoba symbolled by 'C10, C16, C18, C19 and C21', and cultivated in the Cairo-Alexandria desert road were propagated asexually via cutting and *in vitro* culture technique. Two types of explants were tested; shoot tips with 0.5 to 1 cm length and semi-hard nodal segment divided into parts with 1 to 4 cm containing from 1-2 buds after removing whole leaves except small portion to determine cutting side cultivation. Explants were taken from the mid of February, first of March, mid of March, mid of April, first of May and mid of May and at the end of May.

Sterilization protocol

Explants were sterilized after washing with running water for 30 min. then treated with ethyl alcohol of 90 % for 10 sec. followed by a solution of Clorox concentration (15%) +2 drops of Tween 20 for 15 min. and rinsing with distilled sterilized water three times (each time 5 min.).The previous mentioned steps were followed and another step was added; by using mercuric chloride 0.1% for 15 min. and sterile rinsing with distilled water 3 times (5 min. each time).

Culture medium and plant growth regulators

Three different types of culture media were tested on two types of explants (semi hard nodal segment and shoot tips) as follow; (1) MS free hormones, (2) MS + 0.5 mg/l IAA + 1.0 mg/l BA and (3) MS + 0.5 mg/l IBA + 1.0 mg/l NAA. These types of media were supplemented with 25 gm. / L sucrose and 7.5 gm. / L agar and the PH was adjusted to 5.8. The media were autoclaved at 121°C and 1.05 kg cm⁻² for 15 min. The maintained at 26 ± 1°C under 8h light/16 h dark photoperiod under a light intensity of 40 pmol.m⁻².s. A sub-culturing at 4 weeks interval was applied onto fresh medium of the same composition to preserve the cultures.

Vegetative propagation via stem cutting:

Semi hard cuttings were taken in the mid of March from the selected shrubs which were characterized by highest yield. These cuttings were prepared by excised of all leaves except two and

each one of the cutting contains 3-5 nodes. The basal side of cut was done just below the node with 15cm. Basal portion of cutting were dipped for 10 seconds in IBA solution at 3000 ppm and planted in mixture of Peat moss perlite and vermiculite 1:1:1 under mist propagation to study the percentage of rooting / cuttings, number of roots/cutting (Ayerza et al., 1996; Bashir et al., 2001 and Hamad 2010).

Statistical analysis:

Each treatment was performed in six jars containing five explants and each experiment was replicated three times. Data were subjected to analysis of variance by MSTAT-C (1990) Computer statistical analysis program. LSD, test at the 5% level of significance ($P=0.05$)

Results and Discussions:

Explants taking time has a great effect on jojoba plant proliferation, it was noticed that the best time for taking explants begin from the mid of March to mid of April. Moreover, type of tested explants revealed different response towards proliferation (Fig.1), Semi-hard woody stem was the most preferable explants; meanwhile, shoot tips failed to proliferate. In this respect Roussos et al. (1999) used DKW basal medium supplemented with various concentrations of BAP and observed that BAP alone induced 15 shoots per original seedling explants (8 to 10-week-old) over a 12 week period especially when used at a concentration of 4.0 mg L^{-1} . The juvenility of the explants probably contributed to this high proliferation rate.

Effect of jojoba plant genotype and plant growth regulators on proliferation.

Concentrations of different growth regulators played an important role on growth and proliferation of jojoba genotypes. It was clear that combination of BA+NAA (3 mg L^{-1} and 0.5 mg L^{-1} respectively) showed highest rate of multiplication in respect to shoot number, shoot length and leaves number as shown in Table (1). Meanwhile, BA+ NAA at 2 mg L^{-1} and 0.5 mg L^{-1} respectively had least efficiency on shoot number. Combination of 3 mg L^{-1} BA+ 2 mg L^{-1} TDZ yield higher leaves number and shoot length in compare to combination of 2 mg L^{-1} BA+ 0.5 mg L^{-1} NAA. Regarding genotype effect on the proliferation, it was clear that genotype C18 showed almost highest records of vegetative parameters (shoot number and shoot length) irrespective of the tested hormones. On the other hand, C10 and C16 revealed the highest proliferation of shoot numbers. Amr et al., (2013) stated that jojoba genotype 'Al-Maddenah' conveyed the highest response to nodal segments, with MS culture medium supplemented with BAP (3 mg) alone and BAP (3 mg), in combination with IAA (7 mg). Modifying sucrose concentration in

the culture medium (20 g, 40g, 50 g and 60 g) enhanced shoot formation of nodal segments of the genotype ‘Hada Al-Sham’. Concerning shoot tip culture, explants of the genotype ‘Hada Al-Sham’ were cultured on MS+BAP (5 mg)+IAA (5 mg) produced the highest percentages of bud sprouting and shoot formation.

Regarding rooting, proliferated plants failed to initiate roots when different types of hormones (BAP, TDZ and NAA) with different concentration (0.5, 1 and 2 mg/L) were tested. However, the tested genotypes failed to initiate any roots.

Growth regulators Genotype	2.0 BA + 0.5 NAA			3.0 BA + 0.5 NAA			3.0 BA + 2.0 TDZ		
	Shoot number	Leaves number	Shoot length	Shoot number	Leaves number	Shoot length	Shoot number	Leaves number	Shoot length
C10	1.0	4.0	10.0	3.9	4.5	10.90	2.3	4.0	11.90
C16	1.5	2.8	12.80	2.6	4.9	11.90	1.0	4.0	12.20
C18	2.3	3.0	12.60	4.1	3.9	12.80	2.8	2.6	13.80
C19	1.2	3.0	9.20	3.3	2.6	10.10	2.0	2.6	11.80
C21	1.9	3.2	11.10	3.9	3.3	12.70	1.8	4.0	10.70
L.S.D at 0.5	0.12	0.23	1.1	1.4	0.99	2.2	0.12	1.9	2.1

(Table 1): Effect of jojoba plant genotype and plant growth regulators on proliferation.

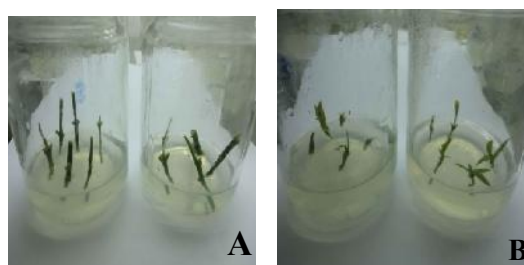


Fig.1: Nodal segments (A) and shoot tip (B) of jojoba used as explants after two weeks

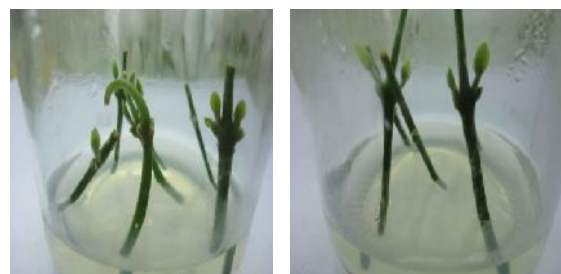


Fig.2: Proliferation of jojoba nodal segments after two weeks



Fig.3: Proliferation of jojoba nodal segments after six weeks.

Characterization of jojoba cutting parameters resulted from vegetative propagation

Rooting parameters were affected by the genotype of the tested strains, e.g., cuttings from strain C18 recorded the highest value of rooting percentage, number of roots, length of primary root and diameter of primary root. Meanwhile, strain C10 showed a lowest record regarding the same parameters of root. On the other hand, C16, C19 and C21 exhibited an intermediate value of root parameters (Table 2).

In this respect, Zhou (2002) related the rooting rates of jojoba cuttings to age of mother plant, temperature and humidity as the rooting rates of cuttings from young and old female jojoba shoots were 60 - 64 and 3-5%, respectively at a temperature of 19-23°C and 80-85% R.H. Cao and Gao (2003) also indicated that the rooting ratio of young jojoba individual was higher than mature individuals. Previously, Lee & Palzkill (1984) reported that clones with a high rooting percentage also tended to produce an increased number of roots per cutting.

Regarding primary shoot length data obtained from strains C10 and C18 detected the lowest and highest value, respectively. However, lowest score of primary shoot diameter was observed by strain C18 and the highest score was observed by strain C21.

It was clear that by increase the rooting percentage the survival percentage increase, similar findings were reported by Luqman *et al.*, (2004) in semi-hardwood cuttings of guava.

Table (2): Growth parameters jojoba cuttings as affected by strain genotype.

Strain No.	Rooting %	No. of roots / cutting	Length of primary root (cm)	Diameter of primary root (mm)	Length of primary shoots (cm)	Diameter of primary shoot (mm)	Survival %
C10	32.1	12.35	5.12	0.34	4.12	1.19	31.1
C16	0	15.36	6.13	0.53	5.18	1.21	37.2
C18	38.1	19.78	8.21	0.71	6.24	1.05	45.1
C19	5	15.12	7.81	0.60	4.23	1.17	36.2
C21	42.3	18.19	5.23	0.45	5.92	1.32	39.4
	4						
	36.1						
	5						
	39.1						
	2						
L.S.D	2.19	1.01	1.19	0.10	2.15	0.32	3.05

5%							
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Fig. (4): Vegetative propagation of jojoba via semi hard stem cuttings in medium comprising peat moss, perlite and vermiculite.

Conclusion

The present investigation proved that propagation via stem cutting treated with IBA at 3000 ppm concentration was the best method for obtaining the highest percentage of rooting in semi-hard wood cuttings of jojoba plants in the rooting medium comprising peat moss, perlite and vermiculite; however, it is difficult to initiate roots via *in vitro* culture technique.

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