

## The Influence of Some Growth Regulators on Proliferation and Rooting *in Vitro* of Propagated *Jojoba*.

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**Abstract:** These procedures were conducted *in vitro* for propagation of *Jojoba*. Shoot tip explants were cultured on MS, WPM and B5 media at different of salt strengths (double full strength, one and half full strength, one and quarter full strength, full strength, 3/4, 1/2, 1/4 and 1/8) free of growth regulators. Effect of MS and 1.25 WPM media supplemented with 2ip, at various concentrations of 0.0, 0.1, 0.2, 0.4, 0.8 and 1.6 mgL<sup>-1</sup>, Kin and IAA at various concentrations of 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mgL<sup>-1</sup> in addition to 0.170 mgL<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric acid and 0.25 mgL<sup>-1</sup> CuSO<sub>4</sub> for each. Also the effect of 1.25 WPM supplemented with BA at concentrations of (0.0, 1.75, 3.5, 7.0 mgL<sup>-1</sup>) in addition to 0.170 mgL<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric acid and 0.25 mgL<sup>-1</sup> CuSO<sub>4</sub> were investigated, to study their effects on the growth and development of *Jojoba* shoots in the multiplication stage. In the rooting stage the effect of IBA at 4.9, 9.9 and 19.9 mgL<sup>-1</sup> when it added to MS full strength and 1.25 WPM full strength media was investigated. Results showed that shoot growth at the establishment stage required 1.25 strength of WPM medium or MS full strength medium (free of growth regulators) and shoot multiplication required MS strength supplemented with 0.1 2ip mgL<sup>-1</sup> + 0.5 mgL<sup>-1</sup> IAA + 2.0 mgL<sup>-1</sup> Kin + 0.170 mgL<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O + 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub> + 12.0 mgL<sup>-1</sup> Boric acid + 0.25 mgL<sup>-1</sup> CuSO<sub>4</sub> or 1.25 full strength of WPM medium supplemented with 7.0 mgL<sup>-1</sup> BA + 0.5 mgL<sup>-1</sup> IAA + 0.170 mgL<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O + 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub> + 12.0 mgL<sup>-1</sup> Boric acid + 0.25 mgL<sup>-1</sup> CuSO<sub>4</sub>. Rooted shoots required MS full strength with 4.9 mgL<sup>-1</sup> IBA. Rooted shoots were acclimatized and successfully transferred to soil (peat: sand 1:2 v/v) with 60 % the surviving of plants.

**Key Words:** *Jojoba* Establishment, Multiplication, Rooting and Acclimatization stage.

### INTRODUCTION

*Simmondsia chinensis* is a desert shrub indigenous to Arizona, California and Northern Mexico. It grows in a number of deserts worldwide including Israel's Negev Desert. A woody evergreen shrub with thick, leathery, bluish-green leaves and dark brown nut like fruit. Male and female flowers are borne on separate plants. The plant can withstand extreme daily fluctuations of temperature. It thrives in well-drained, coarse desert soils and coarse mixtures of gravels and clays, Wisniak, (1977). The mature plants produce about 5 to 10 pounds of seeds, which range between the coffee bean and peanut in size. It is an important forage plant for desert bighorn sheep and mule deer. While birds and rodents eat the seeds, it is toxic to humans and most animals, Office of Arid Lands Studies, (1979). *Jojoba*, *Simmondsia Chinensis* (Link) C. K. Schneid. Family, Jojoba oil has traditionally been used in cosmetics, medicine, and cooking. It appears to alleviate skin irritations and help guard against hair loss. Antifeedant, antiobesity, antioxidant, cosmetic, emetic, laxative, vulnerary. Jojoba oil medicinally used to treat from acne, alopecia, cancer, childbirth, cold, constipation, dermatosis, dysuria, nephrosis, obesity, ophthalmia, poison Ivy, psoriasis, sore and sore throat, wart, wound James, (2002). In recent years, micropropagation has been successful in raising plants *in vitro* to a commercial level in many plant species Chandra and Mishra, (2003). Multiple shoots can be produced *in vitro* and these can be developed into plantlets by regenerating their roots. Thus a single explant source could conceivably provide thousands of new true-to-type plantlets per year. Attempts have been made by several workers to propagate *Jojoba in vitro* on various culture media Agrawal *et al.*, (2002). This study was undertaken to propagate the *Jojoba* through tissue culture technique to increase the number of the female propagule to make the possibility for cultivated it in a wide field area and to have more seeds from it for uses in cosmetic and medicine properties.

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## MATERIALS AND METHODS

This work was carried out in Applied Research Center of Medicinal Plants and Natural Products in Tissue culture Laboratory. National Organization for Drug Control and Research (NODCAR), during the period from 2007- 2009.

### **Plant Material and Explants Source:**

Vegetative shoots were collected from female young plants of *Jojoba*, which grown in pots in the green house of tissue culture laboratory of the Applied Research Center of Medicinal Plants and Natural Products. Shoot tips of 0.5 -1.0 cm in long, were used as source of explants.

These explants were surface sterilized by quick dipping in 70% ethanol containing drops of Twin 20, then sterilized using 0.1% Mg Cl<sub>2</sub> for 5 min after that rinsed for 3 times for 1.0 min in sterilized distilled water for washing prior to transfer to culture medium according to the described method by Kumar *et al.*, (2003).

### **Establishment Stage:**

Shoot tip explants were placed on MS, Murashige and Skoog, (1962), WPM, Woody Plant Lloyed and McCown (1980) and B5, Gamborg *et al* (1968) media at different of salt strengths double full strength, one and half full strength, one and quarter full strength, full strength, 3/4, 1/2, 1/4, and 1/8 free of growth regulators. The pH was adjusted to 5.7 using 0.1N NaOH or 0.1N HCl before autoclaving at 121C° for 20 min and 50 ml of medium were dispensed into culture jars. The explants were cultured on the culture media under laminar air flow hood. Initially, 12 explants were cultured under each treatment per replication, keeping 2 explants in a jar. The cultures were maintained at 25±2C° under 16/8h day photoperiod light condition and darkness respectively from white fluorescent lamps. The experiment was designed in a factorial Completely Randomized Design with 3 replications and 2 factors. The cultures were maintained for 6 weeks in the culture medium before results recorded. Observations were recorded for one month and half to evaluate shoot number/ explants, shoot length (cm), leaf number/ shoot and growth vigor.

### **Multiplication Stage:**

For multiplication induction, excised micro shoots (2.5 cm in length) were transferred to MS full strength and 1.25 WPM supplemented with 2-isopentenyl aminopurine (2ip) at various concentrations of 0.0, 0.1, 0.2, 0.4, 0.8 and 1.6 mgL<sup>-1</sup> and also kinetin (Kin) and indole acetic acid (IAA) at various concentrations of 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mgL<sup>-1</sup> plus the addition of 0.170 mgL<sup>-1</sup> ZnSo<sub>4</sub>.7H<sub>2</sub>o, 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric acid and 0.25 mgL<sup>-1</sup> CuSo<sub>4</sub> for each and also 1.25 strength WPM media supplemented with Benzyl adenine (BA) at concentrations of 0.0, 1.75, 3.5, 7.0 mgL<sup>-1</sup> plus the addition of 0.170 mgL<sup>-1</sup> ZnSo<sub>4</sub>.7H<sub>2</sub>o, 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric acid and 0.25 mgL<sup>-1</sup> CuSo<sub>4</sub>. Two excised shoots were placed in a jar containing 50 ml of culture medium. Shoots were maintained for 6 weeks in the culture medium before results recorded. Observations were recorded for one month and half to evaluate shoots number/ shoot, shoot lengths / shoot (cm), leaf number/ shoot and growth vigor.

### **Rooting Stage:**

For root induction, excised of (*in vitro*) micro shoots (4.0 cm in length) were transferred to solidified MS and 1.25 strength WPM media supplemented with indole butyric acid (IBA) at 4.9, 9.9, and 19.9 mgL<sup>-1</sup>. Two excised shoots were placed in a jar containing 50 ml of culture medium. All the cultures in all 3 stages mentioned before were incubated at 25 ±2C° under 16/8h day photoperiod light condition and darkness respectively from white fluorescent lamps. The obtained shoots were maintained at 6 weeks in the culture media. Observations were recorded for one month to evaluate the shoot number/ shoot, shoot length/ shoot (cm), leaf number/ shoot, root number/ shoot, root length/cm, rooting % and growth vigor. The growth vigor was determined visually according to the morphogenic appearance of the shoots as, 1= dead plants 2 = weak growth, 3 = good growth, 4 = very good growth and 5 = excellent growth according to Bottino, (1981).

### **Acclimatization Stage:**

Rooted shoots; plantlets of (3.5 cm long with 6.0 leaves) which resulted from rooting stage after one month, were washed with tap water to remove the agar from roots before transplanting then planted in plastic pots (12cm diameter) containing peat moss: sand (1:1 and 2:1 v/v), 16 plantlets were used per treatment and kept *in vivo* at a controlled greenhouse under mist system. Observations were recorded after one month and half to evaluate shoot length/ plantlet (cm), leaf number/ plantlet.

**Statistical Analysis:**

The obtained data were pertaining to mean percentage of cultures regeneration. The experiment was implemented in a randomized complete design with four replicates for each treatment. The mean percentage of starting, multiplication and rooting stage were statistically analyzed by Duncan's multiple range test, 1955<sup>6</sup>. The standardized test significant range (L.S.D) at the level of 5 % was used to compare the effect of various treatments according to Snedecor and Cochran., (1982). Data were pertaining to mean percentage of cultures regeneration. The experiment was implemented in a randomized complete design with four replicates for each treatment.

**RESULTS AND DISCUSSION**

**Establishment Stage:**

**1- Effect of MS Salt Strengths:**

1-The shoot tip explants of *Jojoba* were healthy and grew vigorously on MS medium with different salt strengths. Shoot tip explants possessed a significant mean of shoots number 1.35 shoot / explants on MS full strength, also the shoot length was significantly affected by MS full strength. A significant mean of shoot length 3.15cm and a significant average of leaf number 4.45 leaf /shoot was obtained on MS medium full strength, as shown in Table (1). This result in agreement with Wondyifraw and Wannakrairoj (2004) who stated that axillary bud explants of *Aframomum corrorima* (an important culinary and medicinal plant) was developed from the rhizome cultured on MS medium without any growth regulators and MS medium proved to be the best for the establishment stage

**2- Effect of B5 Salt Strengths:**

2- The shoot tip explants of *Jojoba* were healthy and grew vigorously on 1.5 strength B5. Shoot tips explants possessed a significant mean of shoots number 1.0 shoot / explants, also the shoot length and leaf number was significantly affected. Shoot length 1.78 cm and leaf number 4.0 leaf /shoot as shown in Table (2). This result in disagreement with Abou Dahab (2007) who found that using full strength B5 medium led to production of the greatest number of leaves of *Hydrangea macrophylla* Thunb.

**3- Effect of WPM Salt Strengths:**

3- The shoot tip explants of *Jojoba* were healthy and grew very vigorously on 1.25 strength WPM. Shoot tips explants possessed a significant mean of shoots number 2.25 shoot /explants, also the shoot length and leaf number was significantly affected. Shoot length 2.85 cm and leaf number 6.0 leaf /shoot, as shown in Table (3). This result is in respect with Indra and Uppeendra 2004 who found that Woody Plant Medium was found to be the best for the establishment of *Myrica esculenta* buch explants

**Multiplication Stage:**

MS full strength and 1.25 WPM media gave a better growth for the explants of *Jojoba* than 1.5 B5 medium there for the recorded data were taken only for MS and 1.25 WPM.

**1- Effect of MS Full Strength Medium Supplemented with the Following Growth Regulators.**

**1-1-Effect of 2-ip:**

The concentrations of 2ip from 0.1 -1.6 mgL<sup>-1</sup> plus the addition of 0.170 mgL<sup>-1</sup> ZnSo<sub>4</sub>.7H<sub>2</sub>O, 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric acid and 0.25 mgL<sup>-1</sup> CuSo<sub>4</sub> increased the number of shoots. Medium supplemented with 0.1 mgL<sup>-1</sup> 2ip plus 0.170 mgL<sup>-1</sup> ZnSo<sub>4</sub>.7H<sub>2</sub>O 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric acid and 0.25 mgL<sup>-1</sup> CuSo<sub>4</sub> induced a mean of shoot number 2.65 per explants.

No significant effect on shoot length and leaf number among all concentration of 2ip plus other additive and MS control. As shown in Table (4). This result in agreement with Iapichino and Airo (2008) who found that the highest rate of axillary shoot proliferation of *Metrosideros excelsa* was induced on MS agar medium supplemented with 1.96 µm and 1,14 uM IAA after 6 wk in culture.

**1-2-Effect of IAA:**

The concentrations of IAA from 0.5 -2.5 mgL<sup>-1</sup> plus the addition of 0.170 mgL<sup>-1</sup> ZnSo<sub>4</sub>.7H<sub>2</sub>O, 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric acid and 0.25 mgL<sup>-1</sup> CuSo<sub>4</sub> increased the number of shoots and shoot length. The medium supplemented with 0.5 mgL<sup>-1</sup> IAA plus the addition of 0.170 mgL<sup>-1</sup> ZnSo<sub>4</sub>.7H<sub>2</sub>O, 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric acid and 0.25 mgL<sup>-1</sup> CuSo<sub>4</sub> induced a mean of shoots number 1.40 per shoot. Shoot length was

significantly affected by the presence of 0.5 mgL<sup>-1</sup> IAA plus other additive gave a mean of shoot length 5.34 cm, no significant were induced between MS control and all concentration of IAA as shown in Table (5). The synergistic effect of auxin has been demonstrated in many plants Saini (2000).

### **1-3-Effect of Kinetin:**

The concentrations of Kin from 0.5 -2.5 mgL<sup>-1</sup> plus the addition of 0.170 mgL<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric acid and 0.25 mgL<sup>-1</sup> CuSO<sub>4</sub> increased the number of shoots and shoot length. The medium supplemented with 2.0 mgL<sup>-1</sup> Kin plus 0.170 mgL<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric acid and 0.25 mgL<sup>-1</sup> CuSO<sub>4</sub> increased the number of shoots and shoot length. The medium supplemented with 2.0 mgL<sup>-1</sup> Kin plus 0.170 mgL<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric acid and 0.25 mgL<sup>-1</sup> CuSO<sub>4</sub> induced a mean of shoots number 2.29 per shoot. Shoot length was significantly affected by the presence of 2.0 mgL<sup>-1</sup> Kin and other additive produced a mean of shoot length 6.84 cm and also it induced an average of leaf number 8.27 Table (6). This result in respect with Abou Dahab 2007 who mentioned that MS medium plus BA or Kin at rate of 2.0 mgL<sup>-1</sup> were the most effective treatments in given significant increases in shootlet length of *Hydrangea macrophylla* Thunb

### **1-4- Effect of 2ip, IAA and Kin plus the Addition of 0.170 ngL<sup>-1</sup> ZnSo<sub>4</sub>.7h<sub>2</sub>o, 0.170 N<sub>2</sub>h<sub>2</sub>Po<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric Acid and 0.25 MgL<sup>-1</sup> Cuso<sub>4</sub>:**

MS with addition of 0.1 mgL<sup>-1</sup> 2-ip + 0.5 mgL<sup>-1</sup> IAA + 2.0 mgL<sup>-1</sup> Kin plus the addition of 0.170 mgL<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> boric acid and 0.25 mgL<sup>-1</sup> CuSO<sub>4</sub> increased the number of shoots and shoot length. The medium supplemented with 0.1 mgL<sup>-1</sup> 2-ip + 0.5 mgL<sup>-1</sup> IAA + 2.0 mgL<sup>-1</sup> Kin plus other additive induced a mean of shoots number 2.14 per shoot. Shoot length was significantly affected by the presence of all additives. Mean of shoot length was 5.63 cm and it induced average leaf number of 6.12 as shown in Table (7) and Figure (1). This synergistic effect of cytokinin and auxin has been demonstrated in many plants including *Santolina canescens* Casado *et al* (2002) *Bupleurum fruticosum* Fraternal *et al* (2002).

### **2-Effect of 1.25 WPM Supplemented with BA at Concentrations of 0.0, 1.75, 3.5 and 7.0 mgL<sup>-1</sup> plus the Addition of 0.170 mgL<sup>-1</sup> ZnSo<sub>4</sub>.7h<sub>2</sub>o, 0.170 N<sub>2</sub>h<sub>2</sub>Po<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric Acid and 0.25 mgL<sup>-1</sup> CuSo<sub>4</sub> L<sup>-1</sup>:**

2-1- Effect of 1.25 WPM strength medium supplemented with growth regulators 2-ip at concentration of 0.1 -1.6 mgL<sup>-1</sup>, IAA at concentration of 0.5 -2.5 mgL<sup>-1</sup> and Kin at 0.5 -2.5 mg plus the addition of 0.170 mgL<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric acid and 0.25 mgL<sup>-1</sup> CuSO<sub>4</sub> L<sup>-1</sup> did not give any response for the growth of the explants of *Jobba* so the recorded data was taken only for 1.25 WPM supplemented with BA at concentration of 1.75- 7.0 mgL<sup>-1</sup> plus the addition of 0.170 mgL<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 12.0 mgL<sup>-1</sup> Boric acid and 0.25 mgL<sup>-1</sup> CuSO<sub>4</sub>. The medium of 1.25 WPM supplemented with 7.0 mgL<sup>-1</sup> BA plus 0.170 mgL<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O + 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub> + 12.0 mgL<sup>-1</sup> Boric acid + 0.25 mgL<sup>-1</sup> CuSO<sub>4</sub> L<sup>-1</sup> increased the number of shoots, shoot length and leaf number. It induced a mean of shoots number 3.25 per shoot. Shoot length was significantly affected by the presence of all additives. The mean of shoot length was 5.32 cm, and it induced an average of leaf number 8.15 as shown in Table (8) and figure (2). This result in respect with Prakash (2003) who said that BA in combination with different levels of TIBA promoted shoot multiplication in female explants of *Jobba*. Also with agreement with Roussos *et al* (2004), who mentioned that *Jobba* seedling explants were cultured on a modified Driver Kuniyuki medium, supplemented with various concentrations of 6-benzyladenine alone and in combination with silver nitrate. Shoot proliferation was successful at all the concentration tested, with a maximum number of 15, 2 shoots per original explants.

### **2-2- Effect of 1.25 Wpm Supplemented with 7.0 MgL<sup>-1</sup> Ba +0.5 Iaa+.0, 1.75, 3.5 and 7.0 MgL<sup>-1</sup> plus the Addition of 0.170 MgL<sup>-1</sup> Znso<sub>4</sub>.7h<sub>2</sub>o, 0.170 N<sub>2</sub>h<sub>2</sub>po<sub>4</sub>, 12.0 MgL<sup>-1</sup> Boric Acid and 0.25 MgL<sup>-1</sup> Cuso<sub>4</sub> L<sup>-1</sup>:**

The medium of 1.25 WPM supplemented with 7.0 mgL<sup>-1</sup> BA + 0.5 mgL<sup>-1</sup> IAA plus 0.170 mgL<sup>-1</sup> ZnSO<sub>4</sub>.7H<sub>2</sub>O + 0.170 N<sub>2</sub>H<sub>2</sub>PO<sub>4</sub> + 12.0 mgL<sup>-1</sup> Boric acid + 0.25 mgL<sup>-1</sup> CuSO<sub>4</sub> L<sup>-1</sup> increased the number of shoots, shoot length and leaf number. It induced a mean of shoots number 2.45 per shoot. Shoot length was significantly affected by the presence of all additives. Shoot length was 6.32 cm, and it induced an average of leaf number 10.51 as shown in Table (9) and figure (3). This result in agreement with Bashir *et al* (2008), who found that the plant growth regulators combination of 5.55 uM BA + 7.1 uM IAA proved the best for shoot initiation and subsequent growth. Some explants of the *Jobba* genotypes, PKJ-3 and PKJ-6 developed thick rootlets when BA (5.55uM) was used in combination with IBA (6.1uM) or NAA (6.7 uM).

**Rooting Stage:****1-Effect of IBA on the Root Formation of Jojoba Shoots Cultured on MS Medium:**

Individual shoot were excised from 6 weeks old cultures on multiplication medium were placed on MS medium supplemented with 4.9 and 9.9 IBA and 19.9 mg<sup>l</sup><sup>-1</sup>. Shoots induced roots after 30 days. Rooting percentage of 15% of shoots was best achieved by using 4.9 mg<sup>l</sup><sup>-1</sup> IBA Table (10 ) and figure (4). This result was in agreement with Romano *et al*, (2002), who mentioned that the roots of *Ceratonia siliqua* was achieved on growth regulator –free medium after dipping of shoots in indole-3-butyric acid (4.9 mM), and in disagreement with Gita *et al*, 2003<sup>9</sup>, who stated that shoots of *Withania somnifera* (L.) were rooted best (87 %) on MS medium containing 2.0 mg<sup>l</sup><sup>-1</sup> (9.9 uM) indole-3- butyric acid (IBA)

**Table 1:** Effect of MS medium at different of salt strengths on the growth and development of shoot tip of *Jojoba*.

Treatment	Shoots number /explants	Shoot length (cm)	Leaf number /shoot	G.V
2. 0 MS	0. 00 B	0. 00 B	0. 00 B	2. 00 B
1. 5 MS	0. 00 B	0. 00 B	0. 00 B	2. 00 B
1. 25 MS	0. 00 B	0. 00 B	0. 00 B	2. 00 B
MS	1. 35 A	3.15 A	4. 45 A	5. 00 A
¼ MS	0. 00 B	0. 00 B	0. 00 B	2. 00 B
½ MS	0. 00 B	0. 00 B	0. 00 B	2. 00 B
¼ MS	0. 00 B	0. 00 B	0. 00 B	2. 00 B
⅙ MS	0. 00 B	0. 00 B	0. 00 B	2. 00 B

Means within a column followed by the same letter are not significantly different (p=0. 05) according to Duncan's multiple range test. Growth vigor (G.V): 1= dead plants 2=weak growth, 3=good growth, 4=very good growth and 5= excellent growth.

**Table 2:** Effect of B5 medium at different of salt strengths on the growth and development of shoot tip of *Jojoba*.

Treatment	Shoots number /explants	Shoot length (cm)	Leaf number shoot	G.V
2. 0 B5	0. 00 B	0. 00 B	0. 00 B	2. 00 B
1. 5 B5	1. 00 A	1.78 A	4. 00 A	5. 00 A
1. 25 B5	0. 00 B	0. 00 B	0. 00 B	2. 00 B
B5	0. 00 B	0. 00 B	0. 00 B	2. 00 B
¼ B5	0. 00 B	0. 00 B	0. 00 B	2. 00 B
½ B5	0. 00 B	0. 00 B	0. 00 B	2. 00 B
¼ B5	0. 00 B	0. 00 B	0. 00 B	2. 00 B
⅙ B5	0. 00 B	0. 00 B	0. 00 B	2. 00 B

Means within a column followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test. Growth vigor (G.V): 1= dead plants 2=weak growth, 3=good growth, 4=very good growth and 5= excellent growth.

**Table 3:** Effect of WPM medium at different salt strengths on the growth and development of shoot tip of *Jojoba*.

Treatment	Shoots number /explants	Shoot length (cm)	Leaf number /shoot	G.V
2. 0 WPM	0. 00 B	0. 00 B	0. 00 B	2. 00 B
1. 5 WPM	0. 00 B	0. 00 B	0. 00 B	2. 00 B
1. 25WPM	2. 25 A	2. 85 A	6. 00A	5. 00 A
WPM	0. 00 B	0. 00 B	0. 00 B	2. 00 B
¼ WPM	0. 00 B	0. 00 B	0. 00 B	2. 00 B
½ WPM	0. 00 B	0. 00 B	0. 00 B	2. 00 B
¼ WPM	0. 00 B	0. 00 B	0. 00 B	2. 00 B
⅙ WPM	0. 00 B	0. 00 B	0. 00 B	2. 00 B

Means within a column followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test. Growth vigor (G.V): 1= dead plants 2=weak growth, 3=good growth, 4=very good growth and 5= excellent growth

**Table 4:** Effect of MS medium supplemented with different concentrations of 2-isopentenyl aminopurine (2iP) on the growth and development of *Jojoba* shoot cultures.

Treatment	Shoots number /shoot	Shoot length (cm)	Leaf number /shoot	G.V
MS cont	1. 13 B	4. 89 A	6. 15 A	5. 00
0.1mg <sup>l</sup> <sup>-1</sup> 2iP	2. 65A	5. 34 A	6. 56A	5. 00
0. 2 mg <sup>l</sup> <sup>-1</sup> 2iP	1. 47 B	5. 14 A	6. 43 A	5. 00
0. 4 mg <sup>l</sup> <sup>-1</sup> 2iP	1. 43 B	4. 97 A	6. 29 A	5. 00
0. 8 mg <sup>l</sup> <sup>-1</sup> 2iP	1. 39 B	4. 86 A	6.18 A	5. 00
1. 6 mg <sup>l</sup> <sup>-1</sup> 2iP	1. 26 B	4. 75 A	6.12 A	5. 00

Means within a column followed by the same letter are not significantly different ( p=0.05) according to Duncan's multiple range test. Growth vigor (G.V):1= dead plants 2=weak growth, 3=good growth, 4=very good growth and 5= excellent growth.

**Table 5:** Effect of MS medium supplemented with different concentrations of indol-acetic acid (IAA) on the growth and development of *Jojoba* shoot cultures.

Treatment	Shoots number /shoot	Shoot length (cm)	Leaf number /shoot	G.V
MS cont	1. 13 A	4. 89 A	6. 15 A	5. 00
0. 5 mg <sup>l</sup> <sup>-1</sup> IAA	1. 24 A	5. 74A	6. 32 A	5. 00
1. 0 mg <sup>l</sup> <sup>-1</sup> IAA	1. 00 B	4.72 B	6. 28 A	5. 00
1. 5 mg <sup>l</sup> <sup>-1</sup> IAA	1. 00 B	4. 68 B	6. 25 A	5. 00
2. 0 mg <sup>l</sup> <sup>-1</sup> IAA	1. 00 B	4. 65 B	6. 13 A	5. 00
2. 5 mg <sup>l</sup> <sup>-1</sup> IAA	1. 00 B	4. 62 B	6. 00 A	5. 00

Means within a column followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test. Growth vigor (G.V): 1= dead plants 2=weak growth, 3=good growth, 4=very good growth and 5= excellent growth.

**Table 6:** Effect of MS medium supplemented with different concentrations of kinetin (Kin) on the growth and development of *Jojoba* shoot cultures.

Treatment	Shoots number /shoot	Shoot length (cm)	Leaf number /shoot	G.V
MS cont	1. 13 C	4. 89 C	6. 15 C	5. 00
0. 5 mg <sup>l</sup> <sup>-1</sup> kin	1. 15 C	5. 2 4 B	6. 41 B	5. 00
1. 0 mg <sup>l</sup> <sup>-1</sup> kin	1. 19 C	5. 3 6 B	6. 32 B	5. 00
1. 5 mg <sup>l</sup> <sup>-1</sup> kin	1. 26 B	5. 41 B	6. 36 B	5. 00
2. 0 mg <sup>l</sup> <sup>-1</sup> kin	2. 29A	6. 84A	8. 27A	5. 00
2. 5 mg <sup>l</sup> <sup>-1</sup> kin	1. 37 B	5. 43 B	6. 51 B	5. 00

Means within a column followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test. Growth vigor (G.V):1= dead plants 2=weak growth, 3=good growth, 4=very good growth and 5= excellent growth.

**Table 7:** Effect of kinetin (Kin), 2iP and IAA on the growth and development of *Jojoba* shoots cultured on MS medium

Treatment	Shoots number /shoot	Shoot length (cm)	Leaf number /shoot	G.V
MS cont	1. 13 B	4. 89 B	6. 15 B	5. 00
0.1mg <sup>l</sup> <sup>-1</sup> 2iP + 0.5 mg <sup>l</sup> <sup>-1</sup> IAA+ 2.0 mg <sup>l</sup> <sup>-1</sup> Kin+				
0.170 mg <sup>l</sup> <sup>-1</sup> ZnSo <sub>4</sub> .7H <sub>2</sub> O + 0.170 mg <sup>l</sup> <sup>-1</sup> N <sub>2</sub> H <sub>2</sub> PO <sub>4</sub> +				
12.0 mg <sup>l</sup> <sup>-1</sup> boric acid + 0.25 mg <sup>l</sup> <sup>-1</sup> CuSo <sub>4</sub> .	2. 14 A	5. 63 A	6. 12 A	5. 00

Means within a column followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test. Growth vigor (G.V):1= dead plants 2=weak growth, 3=good growth, 4=very good growth and 5= excellent growth.

**Table 8:** Effect of 1.25 WPM supplemented with different concentrations of BA.

Treatment	Shoots number /shoot	Shoot length (cm)	Leaf number /shoot	G.V
1. 25 WPM cont	2. 35 B	3. 45 B	5.79 B	5. 00
1.75 mg <sup>l</sup> <sup>-1</sup> BA	2. 41 B	3. 98 B	6. 38 B	5. 00
3. 5 mg <sup>l</sup> <sup>-1</sup> BA	2. 65 B	4. 27B	6.76 B	5. 00
7. 0 mg <sup>l</sup> <sup>-1</sup> BA	3. 25 A	5. 32 A	8. 15 A	5. 00

Means within a column followed by the same letter are not significantly different (p=0.05) according to Duncan's multiple range test. Growth vigor (G.V): 1= dead plants 2=weak growth, 3=good growth, 4=very good growth and 5= excellent growth.

**Table 9:** Effect of 1.25 WPM supplemented with BA and IAA

Treatment	Shoots number /shoot	Shoot length (cm)	Leaf number /shoot	G.V
1.25 WPM	2. 35 B	3. 45 B	5.79 B	5. 00
7.0 mg <sup>l</sup> <sup>-1</sup> BA + 0.5 mg <sup>l</sup> <sup>-1</sup> IAA+ 0.170 mg <sup>l</sup> <sup>-1</sup> ZnSo <sub>4</sub> .7H <sub>2</sub> O +				
0.170mg <sup>l</sup> <sup>-1</sup> N <sub>2</sub> H <sub>2</sub> PO <sub>4</sub> + 12.0 mg <sup>l</sup> <sup>-1</sup> Boric acid + 0.25 mg <sup>l</sup> <sup>-1</sup> CuSo <sub>4</sub> .	2.45 A	6. 32 A	10. 51 A	5.00

**Table 10:** Effect of IBA concentrations on the root formation of *Jojoba* shoots cultured on MS medium.

Treatment	Root number /shoot	Root length (cm)	Rooting %	G.V
MS cont	0.00	0.00	0.00	5.00
4.9 mg <sup>l</sup> <sup>-1</sup> IBA	2. 13 A	1. 54 A	15 % A	5. 00A
9.9 mg <sup>l</sup> <sup>-1</sup> IBA	0.00	0.00	0.00	5. 00
19.9 mg <sup>l</sup> <sup>-1</sup> IBA	0.00	0.00	0.00	5. 00

Means within a column followed by the same letter are not significantly different (p= 0.05) according to Duncan's multiple range test. Growth vigor (G.V): 1= dead plants 2=weak growth, 3=good growth, 4=very good growth and 5= excellent growth.

**Table 11:** Effect of transplanting medium on growth and development of *Jojoba* plantlets grown under mist in a controlled greenhouse

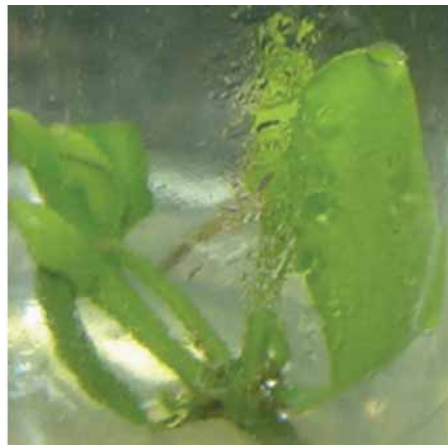
Treatment	Survival %	Leaf number /shoot
Sand:Peat (1:1v/v)	45 %	18.23 B
Sand:Peat (2:1v/v)	60 %	24.15 A

## 2-Effect of IBA Concentrations on the Root Formation of *Jojoba* Shoots Cultured on 1.25 Strength WPM Medium:

Individual shoot were excised from 6 weeks old cultures on multiplication medium were placed on 1.25 strength WPM medium rooting medium. Despite various auxin concentrations of 4.9. 9.9 and 19.9 mg<sup>l</sup><sup>-1</sup> IBA shoots formed no roots *In vitro* but rooted readily when it transferred to a peat and sand substrate *ex vitro*. This result in agreement with Gebhardt and Friedrich (2004), who mentioned that the shoots of *Calluna vulgaris* cv. Did not form any roots *in vitro* but rooted readily if transferred to a peat substrate *ex vitro*.



**Fig. 1:** - Effect of MS + 0.1mg<sup>-1</sup> 2iP + 0.5 mg<sup>-1</sup> IAA+ 2.0 mg<sup>-1</sup>Kin



**Fig. 2:** - Effect of 1.25 WPM + 7. 0 mg<sup>-1</sup> BA



**Fig. 3:** - effect of 1.25 WPM+ 7. 0 mg<sup>-1</sup>IBA + 0.5 mg<sup>-1</sup> IAA



**Fig. 4:** Effect of IBA on the root formation of *Jojoba* cultured on MS medium

1: 4.9 uM IBA

2: 9.9 uM IBA

#### **Acclimatization Stage:**

#### **Effect of Transplanting Medium on Growth and Development of Jojoba Plantlets Grown under Mist in a Controlled Greenhouse:**

After 6 weeks plantlets were transferred to plastic pots (12 diameter), as 60 % of transferred plantlets survived with peat moss and sand (2:1 v/v), Table, 12. This result in respect with Bashir *et al* (2008), the plantlets of Jojoba which developed roots in response to IBA had the maximum survival percentage 63.33 % during acclimatization in the greenhouse as shown in Table (11).

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