

## Enhanced Micropropagation Protocol of Jojoba (*Simmondsia chinensis* L.)

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### ABSTRACT

Jojoba (*Simmondsia chinensis*) is famous for its high-quality fixed oil production with various applications. Micropropagation is an efficient method for in vitro multiplication of plants. Thus, the present study aimed to explore an optimum medium for micropropagation of jojoba. Two experiments were conducted. In the 1<sup>st</sup> experiment, nodal explants of in vitro seedlings cultured on MS medium supplemented with 1,2,4,6,8 or 10  $\mu\text{M}$  of each n<sup>6</sup>-benzyleaminopurine (BAP) or Kinetin (Kin) alone or in combinations (1+1+1, 2+2+1, 4+4+1, 6+6+2, 8+8+2, 10+10+2  $\mu\text{M}$ ) of BAP + Kin + IBA (indol-3-butyric acid) or NAA ( $\alpha$ -naphthalene acetic acid) making up to 24 treatments, after 35 days of initial culture. Shoots obtained on optimum medium (OM) of the 1<sup>st</sup> experiment were selected for further cultivation of nodal (NS) and stub (SS, leftover shoot base 3 mm in size) explants in the 2<sup>nd</sup> experiment. Rooting was achieved  $\frac{1}{2}$  MS+4+4, 6+6, 8+8, 12+12  $\mu\text{M}$  of IBA+NAA. Highest (91.12%) shoot induction with 16.63 number of shoots and 5.22 cm shoot length was obtained in the 1<sup>st</sup> experiment at 4+4+1  $\mu\text{M}$  BA+Kin+IBA, considered as an OM. In the 2<sup>nd</sup> experiment, the rate of shoot induction was 100% from both SN and SS explants at 4 $\mu\text{M}$  BAP+4 $\mu\text{M}$  Kin+1 $\mu\text{M}$  IBA, respectively with 24.45 mean shoots and 6.56 cm shoot length were obtained from nodal explants after 35 days. Year-round data demonstrated 100% shoot induction during April-June on the above-mentioned combination of PGRs. Rooting frequency reached 55.20 %, with an average of 1.86 roots per shoot and 2.45 cm root length in  $\frac{1}{2}$  MS + 12  $\mu\text{M}$  IBA + 12  $\mu\text{M}$  NAA after 65 days. The rooted shoots were successfully acclimatized and grew well in the field.

**Keywords:** Cytokinin, desert gold, jojoba, liquid wax, stub-explants, shoot induction.

### INTRODUCTION

Jojoba is an oil-yielding plant that belongs to the family Simmondsiaceae and is native to the Sonoran Desert of the southwestern USA or northern Mexico (Hassan, 2003). Jojoba is a dioecious plant predominantly found on desert sandy soils. Generally, the soil in which Jojoba grows is neutral to alkaline and rich in phosphorus content. Because of its deep rooting system, it cannot grow in waterlogged and

hardened soils (Bashir *et al.*, 2007). Jojoba has been recognized to produce expensive and golden oil: the plant can be grown on wasteland and desert soil conditions.

Due to its growth habit and being a wonderful source of golden oil, it is so-called “liquid gold from the desert” (Heikrujam *et al.*, 2014).

Jojoba oil is a common component of many cosmetics due to its skin infiltration property (Heikrujam *et al.*, 2014). The popularity of jojoba oil is growing all over the world because it can be utilized in a fantastic variety of applications. As more individuals throughout the world seek out natural and environmentally friendly skin care products, there has been an increase in the demand for jojoba oil (Shah *et al.*, 2020). This is especially true as the number of consumers purchasing organic beauty products continues to rise. As a result of its ability to hydrate, reduce inflammation, and slow down the ageing process, jojoba oil is frequently utilized in the cosmetics industry (Aizenberg *et al.*, 2021). People

### Article History

Received: [January 29, 2025](#), Accepted: [April 24, 2025](#),

Published: [May 13, 2025](#)



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are utilizing jojoba oil in increasing numbers because they are aware of its ability to treat acne and improve the condition of their hair (Naumann, 2022). As the demand for jojoba oil continues to climb, major manufacturers such as the United States and India are expected to increase their production and exports of the product, according to the World Market Report 2023 (World Market Report, 2023).

The propagation of jojoba is hindered due to its recalcitrant nature and slow growth. Low seed viability is also an impediment to rapid multiplication under field conditions. Plant Tissue Culture is an alternative and highly prized for the provision of true-to-type clonal plant production. For this purpose, plant growth regulators (PGR) are used to corroborate the initiation of gene regulation for prompt response of *in vitro* shoot multiplication from cultured plant tissues (Bashir *et al.*, 2008). N<sup>6</sup>-benzyladenine (BA) and N<sup>6</sup>-furfuryladenine (Kinetin) are widely used cytokinins for micropropagation of different plant species (Akram and Aftab, 2008; 2009; 2012a; 2012b). BA promotes axillary shoot growth and multiplication of different recalcitrant plants. To increase its efficacy for rapid shoot multiplication, the addition of a low concentration of indol-3-butyric acid (IBA) or  $\alpha$ -naphthalene (NAA) in the medium is recommended (Ozdemir *et al.*, 2014). Moreover, the same auxins are potent in inducing rooting, which is a key phase for effective micropropagation (Dewir *et al.*, 2016). Besides, jojoba faces serious problems in producing adventitious rooting *in vitro*.

The commercial significance of liquid wax endorses the need for an effective and improved micropropagation system for high-quality oil-producing clones of jojoba. Cell suspension culture is another way for *in vitro* manipulation of jojoba callus to get desired and improved compounds (Akram and Aftab, 2021). Alyousif *et al.*, (2016) did micropropagation for *in vitro* selection to get salt-tolerant plants by successive subculturing of jojoba microshoots. Genotype and sex-specific protocols for *in vitro* micropropagation have been demonstrated for *in vitro* conservation and multiplication of jojoba (Prakash, 2023). Many other reports also demonstrated successful micropropagation and further multiplication of by using different explants (Mills *et al.*, 2004; Bala *et al.*, 2015; Bekheet *et al.*, 2016). Thus, the present study aimed to explore different plant growth regulators for micropropagation and production of a large number of multiple shoots from nodal and 'stub' explants for the establishment of commercially expandable projects.

## MATERIALS AND METHODS

Mature seeds were cleaned with fresh tap water for 10 min, disinfected with 70% ethanol (Merck, Germany) for 30 sec, and sterilized with 15% commercial bleach

(Robin, Reckitt Benckiser, Pakistan, Ltd.) for another 15 min. Finally, seeds were washed with autoclaved distilled water to remove the traces of chlorine and inoculated on the medium. All media were solidified with 0.8% agar (Merck, Germany) after adjusting the pH (5.80) and autoclaved at 104 kPa and 121 °C for 15 min and stored under the culture room conditions of 16 h photoperiod ( $35 \mu\text{mol m}^{-2}\text{s}^{-1}$ ) provided by white tube lights (Philips) and  $25 \pm 2$  °C temperature until use.

Sterilized seeds were cultured on modified MS (Murashige and Skoog, 1962) semi-solid medium (Table 1) containing 1% sucrose under the culture room conditions. The developing seedlings (Fig. 1A) were allowed to grow further up to 60 days to obtain the maximum length to obtain the nodal explants.

Sixty-day-old *in vitro* seedlings were used as initial source material for axillary shoot proliferation and multiplication of jojoba. Different treatments of plant growth regulators (PGRs) were used to find out the best one that formed vigorous and fast growth. Two experiments were conducted for optimization of the medium.

In the 1<sup>st</sup> experiment, nodal explants were cultured on MS medium supplemented with 1, 2, 4, 6, 8 or 10  $\mu\text{M}$  of each n<sup>6</sup>-benzyleaminopurine (BAP) or Kinetin (Kin) alone or in combinations with each other and additionally with indol-3-butyric acid (IBA 1  $\mu\text{M}$ ) or  $\alpha$ -naphthalene acetic acid (NAA 1  $\mu\text{M}$ ), i.e., 1+1+1, 2+2+1, 4+4+1, 6+6+2, 8+8+2, 10+10+2  $\mu\text{M}$  BAP + Kin + IBA or NAA making up to 24 treatments. After 35 days of initial culture, the vigorous growth of *in vitro* shoots was obtained on MS + BAP (4  $\mu\text{M}$ ) + Kin (4  $\mu\text{M}$ ) + IBA (1 $\mu\text{M}$ ), was selected as an optimum medium (OM).

Table 1. Formulation of modified MS Medium

Ingredients	MS medium
<b>a) Macronutrients</b>	<b>mg/L</b>
(NH <sub>4</sub> )NO <sub>3</sub>	1750
KNO <sub>3</sub>	2000
CaCl <sub>2</sub> .2H <sub>2</sub> O	440
MgSO <sub>4</sub> .7H <sub>2</sub> O	370
KH <sub>2</sub> PO <sub>4</sub>	170
<b>b) Micronutrients</b>	<b>mg/L</b>
MnSO <sub>4</sub> .4H <sub>2</sub> O	25.3
ZnSO <sub>4</sub> .7H <sub>2</sub> O	8.6
H <sub>3</sub> BO <sub>3</sub> .7H <sub>2</sub> O	6.2
KI	0.83
Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O	0.25
CuSO <sub>2</sub> .5H <sub>2</sub> O	0.025
CoCl <sub>2</sub> .6H <sub>2</sub> O	0.025
<b>c) Vitamins</b>	<b>mg/L</b>
Glycine	5.0
Nicotinic acid	0.5

Pyridoxine HCl	0.5
Thiamine HCl	0.1
<b>d) Iron</b>	<b>mg/L</b>
Na <sub>2</sub> EDTA.2H <sub>2</sub> O	36.2
FeSO <sub>4</sub> .7H <sub>2</sub> O	27.8
<b>e) Myo-inositol</b>	200 mg/L
f) Sucrose	10 g/L
g) Agar	8 g/L
h) pH 5.80	

The process of *in vitro* rooting of shoots was carried out by culturing 1-2 cm long randomly selected shoots on ½ MS medium supplemented with 4 + 4, 6 + 6, 8 + 8, 12 + 12 µM of IBA + NAA. The data were recorded for the rooting percentage, number, and length of the roots per shoot growing on OM were selected for the preparation of nodal as well as stub explants in the 2<sup>nd</sup> experiment. *In vitro* developing shoots of 1<sup>st</sup> experiment were excised, and the leftover portion of the shoot base (3mm in size) was considered as a 'stub'. Both explants were then cultured in the media as were used in the 1<sup>st</sup> experiment. The data for percent shoot induction, number, and length of shoot (cm) were recorded after 35 days of initial culture. Moreover, the same data was also taken during different months over the year by culturing both types of explants on OM.

The process of *in vitro* rooting of shoots was carried out by culturing 1-2 cm long randomly selected shoots on ½ MS medium supplemented with 4 + 4, 6 + 6, 8 + 8, 12 + 12 µM of IBA + NAA. The data were recorded for the rooting percentage, number, and length of the roots per shoot after 65 days. Rooted shoots were taken out from the culture tubes, rinsed the rooting

zone with clean water, and decontaminated by immersing them in a 0.1% solution of Cabrio® fungicide (FMC, Germany). The treated rooted shoots were then planted in polystyrene cups filled with perlite and incubated under the lab conditions. Irrigation was done with one-fourth strength of MS macro salts once a week. Cups were covered to reduce humidity for 1 week and then let them grow without covering. After 30 days, the plantlets were shifted to a 1:1 ratio of soil mixture (silt + compost) in polythene bags under glasshouse conditions of 25 ± 5 °C temperature and 80% relative humidity.

#### Data Analysis

Data were subjected to analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) at P≤0.05 to check variations in mean values. Before analysis, the assumptions were checked for homogeneity of variance. The differences between mean values were represented by standard error (±). Each treatment had three replicates, and the experiment was repeated thrice. Statistical software SPSS v. 12.0 was used for analysis and interpretation of results.

## RESULTS AND DISCUSSION

### Shoot Induction

The first experiment of the present study demonstrated that all treatments induced shoots from nodal explants of germinated seedlings of jojoba (Fig. A) with different responses of shoot induction, number, and shoot length (Table 2). The shoot induction percentage was significantly improved with the increasing levels of PGRs. Generally, all concentrations of BAP were more effective and produced 90.41% shoot induction at 10 µM as compared to Kin.

**Table 2.** Effect of different levels of plant growth regulators on *in vitro* shoot growth of jojoba from nodal explants of *in vitro* germinated seedling (1<sup>st</sup> experiment) after 35 days of initial culture.

PGRs (µM)	Shoot induction (%)	Shoot number	Shoot length (cm)
1 BAP	75.70±5.55 <sup>d</sup>	1.35±0.20 <sup>ef</sup>	0.85±0.05 <sup>ef</sup>
2	78.52±4.63 <sup>d</sup>	1.56±0.60 <sup>ef</sup>	0.90±0.44 <sup>ef</sup>
4	82.41±4.44 <sup>c</sup>	1.04±0.63 <sup>ef</sup>	2.41±0.41 <sup>cd</sup>
6	85.25±4.40 <sup>b</sup>	1.00±0.23 <sup>efg</sup>	2.33±0.88 <sup>cd</sup>
8	86.36±7.44 <sup>b</sup>	2.45±0.74 <sup>def</sup>	2.22±1.63 <sup>cd</sup>
10	90.41±6.45 <sup>ab</sup>	2.65±0.41 <sup>def</sup>	3.33±1.41 <sup>bc</sup>
1 Kin	55.44±8.45 <sup>de</sup>	0.33±0.0.22 <sup>efg</sup>	0.45±0.11 <sup>ef</sup>
2	63.56±4.12 <sup>ef</sup>	0.44±0.22 <sup>efg</sup>	0.81±0.71 <sup>ef</sup>
4	68.41±4.52 <sup>de</sup>	0.45±0.12 <sup>efg</sup>	2.44±1.11 <sup>cd</sup>
6	69.32±3.25 <sup>de</sup>	1.25±0.45 <sup>ef</sup>	0.74±1.33 <sup>ef</sup>
8	70.52±6.85 <sup>de</sup>	1.52±0.61 <sup>ef</sup>	0.15±0.55 <sup>efg</sup>
10	77.22±9.24 <sup>d</sup>	1.45±0.85 <sup>ef</sup>	0.88±0.66 <sup>ef</sup>
<b>BAP + Kin + IBA</b>			
1+1+1	78.45±4.12 <sup>d</sup>	4.44±1.45 <sup>de</sup>	2.15±1.44 <sup>cd</sup>
2+2+1	81.41±3.45 <sup>c</sup>	7.45±2.45 <sup>bc</sup>	2.55±1.05 <sup>cd</sup>
4+4+1	91.12±4.52 <sup>a</sup>	16.63±4.25 <sup>a</sup>	5.22±4.25 <sup>a</sup>
6+6+2	88.44±6.52 <sup>b</sup>	11.41±4.32 <sup>b</sup>	4.44±1.22 <sup>b</sup>

8+8+2	86.41±4.44 <sup>b</sup>	09.45±4.33 <sup>bc</sup>	4.25±2.22 <sup>b</sup>
10+10+2	87.85±6.74 <sup>b</sup>	05.21±3.41 <sup>d</sup>	3.33±1.89 <sup>bcd</sup>
<b>BAP + Kin + NAA</b>			
1+1+1	55.41±3.23 <sup>g</sup>	2.47±0.82 <sup>def</sup>	2.11±0.88 <sup>cde</sup>
2+2+1	57.55±3.33 <sup>eg</sup>	1.45±0.32 <sup>ef</sup>	2.22±0.94 <sup>cd</sup>
4+4+1	60.45±4.12 <sup>fg</sup>	1.22±0.74 <sup>ef</sup>	2.55±1.11 <sup>cd</sup>
6+6+2	66.45±4.32 <sup>ef</sup>	1.22±0.41 <sup>ef</sup>	3.65±1.12 <sup>bc</sup>
8+8+2	68.04±2.85 <sup>ef</sup>	1.15±0.18 <sup>efg</sup>	3.33±1.44 <sup>bc</sup>
10+10+2	68.45±4.65 <sup>ef</sup>	2.44±3.32 <sup>def</sup>	3.33±1.22 <sup>bc</sup>

Mean values (±SE) followed by different letters are significantly different according to Duncan's multiple range test (DMRT)  $P \leq 0.05$ . Each treatment has three replicates, and the experiment was repeated thrice.

Despite that, when both cytokinins of BAP and Kin were combined (BAP + Kin) along with 1 µM IBA in MS medium, the frequency of shoot formation was significantly improved. Amongst all treatments, BA+Kin+IBA (4+4+1 µM) was the best obtained the highest 91.12% shoot induction with 16.63 mean shoots and 5.22 shoot length after 35 days of initial culture. Other combinations of BA+Kin+NAA were less effective, albeit they produced shoots in all culture vessels (Table 2). The shoots with BA+Kin+IBA were healthy and green, whereas the shoots were

stunted and yellowish-green in their combination with NAA after the same culture period.

In the 2<sup>nd</sup> experiment, shoot induction was observed on all treatments with varied degrees of response. The highest (100%) shoot induction was obtained with SN as well as SS on MS medium supplemented with 4 µM BAP + 4 µM Kin + 1 µM IBA after 35 days of initial culture (Table 3). The highest number of shoots (24.45) and 6.56 cm shoot length was obtained on the same medium from nodal explants (Fig. 1 B, C, D).

**Table 3.** Effect of different levels of plant growth regulators on *in vitro* shoot formation of jojoba from nodal and stub explants (2<sup>nd</sup> experiment) after 35 days of initial culture.

PGRs (µM)	Shoot formation from node (SN)			Shoot formation from stubs (SS)		
	Percent value	Shoot number	Shoot length (cm)	Percent value	Shoot number	Shoot length (cm)
1 BAP	88.12±4.44 <sup>bc</sup>	1.25±0.50 <sup>cde</sup>	0.55±0.02 <sup>de</sup>	78.25±8.11 <sup>cde</sup>	0.85±0.33 <sup>efg</sup>	1.25±0.91 <sup>ef</sup>
2	88.25±4.21 <sup>bc</sup>	1.56±0.60 <sup>cde</sup>	0.85±0.33 <sup>de</sup>	88.36±6.66 <sup>bc</sup>	0.95±0.33 <sup>efg</sup>	1.85±0.90 <sup>de</sup>
4	92.45±5.55 <sup>b</sup>	2.45±0.71 <sup>cd</sup>	1.25±0.88 <sup>cde</sup>	82.55±5.55 <sup>bcd</sup>	1.25±0.77 <sup>def</sup>	2.36±1.11 <sup>cd</sup>
6	92.14±5.55 <sup>b</sup>	1.26±0.52 <sup>de</sup>	1.45±0.91 <sup>cde</sup>	82.45±7.25 <sup>bcd</sup>	1.63±0.85 <sup>def</sup>	2.52±1.14 <sup>cd</sup>
8	95.36±5.55 <sup>ab</sup>	1.85±0.41 <sup>cde</sup>	1.05±0.95 <sup>de</sup>	85.35±8.88 <sup>bcd</sup>	1.72±0.87 <sup>def</sup>	1.71±0.91 <sup>de</sup>
10	96.25±8.21 <sup>ab</sup>	1.98±0.61 <sup>cde</sup>	1.71±0.95 <sup>cde</sup>	91.15±10.11 <sup>bc</sup>	1.10±0.50 <sup>ef</sup>	1.02±0.91 <sup>ef</sup>
1 Kin	65.41±6.22 <sup>de</sup>	0.25±0.12 <sup>ef</sup>	0.55±0.21 <sup>de</sup>	58.11±5.55 <sup>fg</sup>	0.45±0.11 <sup>efg</sup>	0.55±0.11 <sup>fg</sup>
2	70.30±3.33 <sup>cde</sup>	0.56±0.12 <sup>ef</sup>	0.85±0.77 <sup>d</sup>	68.33±6.18 <sup>ef</sup>	0.96±0.41 <sup>fg</sup>	0.65±0.22 <sup>fg</sup>
4	71.40±3.24 <sup>cde</sup>	0.45±0.02 <sup>ef</sup>	2.25±1.00 <sup>bc</sup>	77.55±7.77 <sup>de</sup>	1.75±0.99 <sup>def</sup>	0.86±0.33 <sup>fg</sup>
6	72.32±4.24 <sup>cde</sup>	1.05±0.55 <sup>def</sup>	0.45±0.45 <sup>de</sup>	82.45±8.11 <sup>bcd</sup>	1.33±0.95 <sup>def</sup>	1.03±0.78 <sup>efg</sup>
8	75.15±4.48 <sup>cd</sup>	1.09±0.80 <sup>def</sup>	0.05±0.01 <sup>e</sup>	78.44±7.15 <sup>cde</sup>	1.66±0.89 <sup>def</sup>	1.11±0.83 <sup>ef</sup>
10	74.10±4.28 <sup>cd</sup>	1.03±0.71 <sup>def</sup>	0.71±0.07 <sup>de</sup>	75.10±5.55 <sup>def</sup>	1.11±0.88 <sup>ef</sup>	1.41±0.90 <sup>def</sup>
<b>BAP + Kin + IBA</b>						
1+1+1	80.88±3.50 <sup>bcd</sup>	3.33±1.00 <sup>cd</sup>	1.25±0.88 <sup>cde</sup>	95.25±9.11 <sup>b</sup>	3.45±1.22 <sup>cde</sup>	4.12±1.44 <sup>b</sup>
2+2+1	82.23±6.33 <sup>bcd</sup>	6.12±1.25 <sup>cd</sup>	2.22±0.95 <sup>bc</sup>	95.66±9.11 <sup>b</sup>	5.55±2.11 <sup>c</sup>	2.22±1.02 <sup>cde</sup>
4+4+1	100 <sup>a</sup>	24.45±3.25 <sup>a</sup>	6.56±2.33 <sup>a</sup>	100 <sup>a</sup>	10.53±3.33 <sup>a</sup>	5.44±2.33 <sup>a</sup>
6+6+2	89.26±4.85 <sup>b</sup>	13.25±2.45 <sup>bc</sup>	5.22±1.44 <sup>ab</sup>	89.45±8.11 <sup>bc</sup>	9.25±2.50 <sup>ab</sup>	3.33±1.33 <sup>c</sup>
8+8+2	95.25±3.33 <sup>ab</sup>	11.25±2.11 <sup>bc</sup>	4.25±1.11 <sup>b</sup>	69.12±6.11 <sup>ef</sup>	8.12±2.11 <sup>bc</sup>	5.23±1.78 <sup>ab</sup>
10+10+2	95.65±3.33 <sup>ab</sup>	7.65±1.44 <sup>cd</sup>	2.13±0.99 <sup>bcd</sup>	70.14±7.11 <sup>ef</sup>	8.45±2.24 <sup>bc</sup>	4.44±1.25 <sup>b</sup>
<b>BAP + Kin + NAA</b>						
1+1+1	60.18±4.44 <sup>def</sup>	1.81±0.91 <sup>cde</sup>	1.44±0.98 <sup>cde</sup>	65.15±6.12 <sup>efg</sup>	3.12±1.33 <sup>cde</sup>	1.05±0.91 <sup>ef</sup>
2+2+1	66.44±2.22 <sup>de</sup>	2.52±0.98 <sup>cd</sup>	1.11±0.98 <sup>cde</sup>	66.66±6.66 <sup>efg</sup>	4.44±1.44 <sup>cd</sup>	1.85±0.95 <sup>cde</sup>
4+4+1	70.25±3.33 <sup>cde</sup>	2.66±0.98 <sup>cd</sup>	2.32±1.02 <sup>bc</sup>	72.25±9.29 <sup>def</sup>	3.53±1.33 <sup>cd</sup>	1.63±0.95 <sup>def</sup>
6+6+2	70.71±3.33 <sup>cde</sup>	1.78±0.75 <sup>cde</sup>	4.21±1.14 <sup>b</sup>	79.77±8.28 <sup>cde</sup>	5.44±2.11 <sup>c</sup>	1.95±0.96 <sup>cde</sup>
8+8+2	72.25±4.24 <sup>cd</sup>	1.45±0.88 <sup>de</sup>	2.40±1.05 <sup>bc</sup>	79.55±9.99 <sup>cde</sup>	3.22±1.11 <sup>cde</sup>	2.25±1.33 <sup>cd</sup>
10+10+2	72.56±4.74 <sup>cd</sup>	5.65±2.33 <sup>cd</sup>	2.33±1.11 <sup>bc</sup>	80.26±8.88 <sup>cde</sup>	4.74±1.12 <sup>cd</sup>	2.45±1.44 <sup>cd</sup>

Mean values ( $\pm$ SE) followed by different letters are significantly different according to Duncan's multiple range test (DMRT)  $P \leq 0.05$ . Each treatment has three replicates, and the experiment was repeated thrice.

The shoot induction response of the 2<sup>nd</sup> experiment was not much different as compared to 1<sup>st</sup> experiment. All concentrations of either PGR developed shoots. The shoot growth on kin was poor whereas BAP developed greenish and healthy shoots. The combined

effect of all three PGRs (BAP+Kin+IBA) was more pronounced at their higher concentration levels (Table 3) as compared to the combined effect of cytokinins with NAA.



**Fig 1.** Micropropagation of jojoba on MS medium supplemented with various growth regulators. A) *In vitro* seed germination on MS medium of 15-day-old. B, C, D) Shoot formation from initial bud culture directly from axenic seedlings at 4  $\mu$ M BAP + 4  $\mu$ M Kin + 1  $\mu$ M IBA, respectively, after 35 days. E, F) *In vitro* shoot formation from stubs (SS) at 4  $\mu$ M BAP + 4  $\mu$ M Kin + 1  $\mu$ M IBA after the same culture period. G) 60 days old elongating shoot on the medium as described in F. H) Rooted shoot at  $\frac{1}{2}$ MS + 12  $\mu$ M IBA + 12  $\mu$ M NAA after 65 days of culture. I) Hardening and acclimatization of plantlets in soil under the glasshouse conditions.

The values of the same parameters were lower with stubs, i.e., 10.53 number of shoots and 5.44 cm shoot length on the same treatments and incubation period (Fig. 1E, F). Such shoots were elongated to a culturable length after 60 days (Fig. 1G). The rate of shoot induction was fair, whereas shoot number and length were lower from nodal and stub explants obtained with 10  $\mu$ M Kin.

#### Effect of Time of Year

The month-wise data demonstrated that April-June was the best month to obtain 100% shoot induction with a 29.56 mean number and 5.55 cm length from nodal explants (Table 4). Cytokinins regulate the synthesis of proteins necessary for adventitious shoot formation and the stimulation of cell division, and they additionally play a vital role in shoot regeneration.

**Table 4.** Effect of different periods on shoot formation of Jojoba on the optimum level of BAP (4  $\mu$ M) + Kin (4  $\mu$ M) + IBA (1 $\mu$ M) in MS medium.

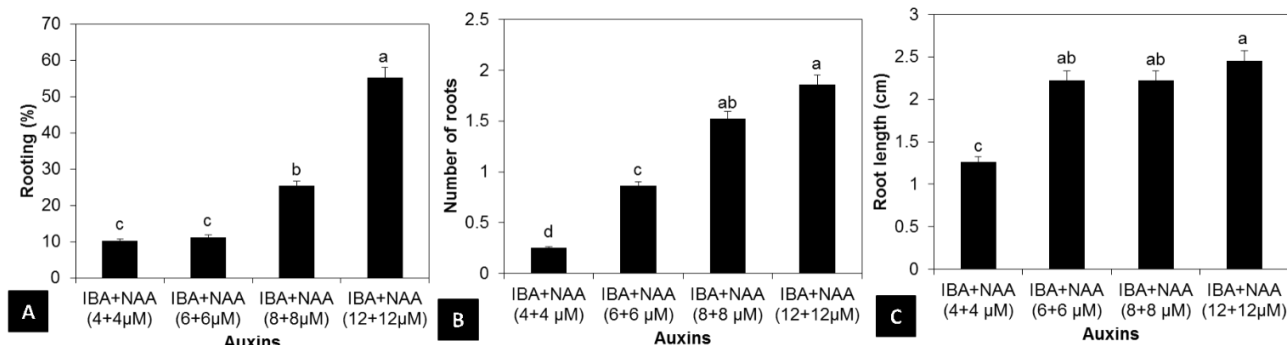
Period	Shoot formation from node (SN)			Shoot formation from stubs (SS)		
	Percent value	Number	Length (cm)	Percent	Number	Length (cm)
Jan-Mar	95.50 $\pm$ 11.11 <sup>b</sup>	27.77 $\pm$ 5.77 <sup>b</sup>	4.26 $\pm$ 1.55 <sup>b</sup>	90.81 $\pm$ 13.15 <sup>b</sup>	20.22 $\pm$ 4.44 <sup>ab</sup>	3.26 $\pm$ 1.11 <sup>ab</sup>
Apr-Jun	100 <sup>a</sup>	29.56 $\pm$ 6.66 <sup>a</sup>	5.55 $\pm$ 2.55 <sup>a</sup>	100 <sup>a</sup>	21.31 $\pm$ 5.55 <sup>a</sup>	3.85 $\pm$ 1.15 <sup>a</sup>
Jul-Sep	91.26 $\pm$ 13.15 <sup>bc</sup>	26.33 $\pm$ 6.66 <sup>bc</sup>	4.25 $\pm$ 3.33 <sup>b</sup>	92.15 $\pm$ 14.11 <sup>b</sup>	18.12 $\pm$ 6.11 <sup>c</sup>	3.05 $\pm$ 1.16 <sup>ab</sup>
Oct-Dec	89.62 $\pm$ 15.10 <sup>c</sup>	20.36 $\pm$ 5.33 <sup>c</sup>	4.06 $\pm$ 2.55 <sup>b</sup>	88.36 $\pm$ 08.88 <sup>bc</sup>	18.00 $\pm$ 6.15 <sup>c</sup>	3.45 $\pm$ 1.20 <sup>ab</sup>

Mean values ( $\pm$ SE) followed by different letters are significantly different according to Duncan's multiple range test (DMRT)  $P \leq 0.05$ . Each treatment has three replicates, and the experiment was repeated thrice.

Llorente and Apóstolo (1998) examined various growth regulators for jojoba micropropagation. They found that at a concentration of 1 mg/L BAP, 4.6-fold shoots were produced. By cultivating nodal explants of jojoba on MS media containing 4.44  $\mu$ M BAP and 74  $\mu$ M adenine, the shoot number was increased from 10 to 15 when the plants were sub-cultured (Singh *et al.*, 2008). These reports lend credence to our findings. Because it increases the manufacture of other precursor chemicals, including zeatin, BAP is necessary for increased shoot induction in jojoba, as evident by previous reports (Singh *et al.*, 2008).

Our results demonstrated that sub-culturing of shoots on the fresh medium improved the number and length of shoots. Shoot induction frequency may be increased by using auxins (IAA, NAA or IBA) along with cytokinins in the shoot induction medium. Hassanein *et al.* (2015) reported sprouting and multiplication

from nodal segments of 22 years old jojoba with low concentration of NAA (0.01mg/L) + BAP (3 mg/L). Hassan (2003) used encapsulated buds of jojoba and obtained the highest shoot induction on MS + 1 mg/L BAP + 40 mg/L adenine + 3 mg/L IAA. Similarly, the highest 14.44 mean number of shoots with 4.78 cm long shoots has been reported in jojoba at 2 mg/L BAP + 0.2 mg/L NAA (Bala *et al.*, 2015). Bekheet *et al.* (2016) reported 3.5 cm-long *in vitro* shoots of jojoba after six weeks. Apart from these reports using BAP + auxins, very low concentration of sole TDZ has also been reported as a strong cytokinin for high number of *in vitro* shoot multiplication of various plants (Huetteman, and Preece, 1993), and in jojoba (Bala *et al.*, 2019) obtaining 4.57 the highest mean number of shoots which is relatively less than the number 24.45 of our results with 4  $\mu$ M BAP + 4  $\mu$ M Kin + 1  $\mu$ M IBA from nodal explants.



**Fig. 2.** Rooting of *in vitro* shoots of Jojoba recorded after 65 days of culture. A) Rooting percentage. B) Number of roots. C) Root length. Bars over columns are the  $\pm$ SE of the means. Small alphabetical letters over the columns indicate significantly different results according to Duncan's multiple range test (DMRT)  $p \leq 0.05$ .

These reports showed the importance of auxin and BAP for improved shoot growth in jojoba. Similarly, one of our articles on teak signifies the effect of IBA for axillary shoot regeneration from nodal explants (Bashir *et al.*, 2008). In the current research, we used a new stub explant material with an improved rate of shoot multiplication parallel to the nodal explant of jojoba.

**Rooting and Acclimatization** The rate of rooting was significantly improved by increasing the concentration of auxin in the medium until 65 days of culture (Fig. 2). The highest rooting (55%) was obtained at 12  $\mu$ M IBA + 12  $\mu$ M NAA (Fig. 2A) with a 1.8 mean number (Fig. 2 B) and 2.4 cm root length (Fig. 2 C) after 65 days (Fig. 1H). Similar results have been reported (Shah *et al.*, 2020). On the other hand, low auxin concentration (4  $\mu$ M IBA + 4  $\mu$ M NAA) was less effective for obtaining the same rooting

parameters. Upon culturing, such micro-cuttings produced axillary shoots and elongated. This phenomenon is supported by the hypothesis that the reserve cytokinins in the prior cultured tissues do the same, which may also be influenced by an elevated temperature condition (Luqman *et al.*, 2004). The present study opposed the idea that IBA alone did not affect root length considerably, as supported by Palzkill's viewpoint (Palzkill, 1988). Moreover, we observed that root length increases by increasing IBA in the medium. On the other hand, Zhou (2002) achieved the highest rooting at a very low concentration of auxin, like Bashir *et al.* (2008) and Singh *et al.* (2008). Rooting of jojoba shoots depends upon the physical and chemical factors, including low salt levels in the medium. For that reason, low salt

concentration was known to enhance the rooting frequency of micro-shoots in jojoba (Kumar and Mangal, 2009).

The present study demonstrates an efficient and reproducible method of micropropagation of jojoba using stub explants as a new explant material on the modified MS medium in April-June. High-frequency multiple number (24.45) of shoot formation was possible using a combination of plant growth regulators of BAP + Kin + IBA (4+4+1  $\mu$ M) from nodal explants of jojoba. The rooting of *in vitro* shoots was subsequently achieved with the successful acclimatization of the plantlets under field conditions. The acclimatized plants in the field showed vigorous growth compared to the mother plants.

### CONFLICT OF INTEREST

The authors declared no conflict of interest.

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