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In Vitro Propagation of Jojoba [*Simmondsia chinensis* (Link) Schneider] through Nodal Segments of Female Plants

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Abstract: Nodal segments from first, second and third nodes (from the tip of branch) of three mature female plants of jojoba [*Simmondsia chinensis* (Link) Schneider] were cultured on MS medium supplemented with different concentrations of BAP (1.0 to 5.0 mg L^{-1}). Number of days required for bud break ranged from 7-13 with 5.0 mg L^{-1} BAP. Increasing BAP concentration from 1.0 to 4.0 mg L^{-1}, axilliary shoot bud proliferation/multiplication percentage increased. Second and third nodes exhibited better multiple shoot formation (32.75%, 28.0%, respectively) as compared to the first node (15.52%) and the differences were significant. Shoot proliferation was successful at all the concentrations tested with a maximum number of 14 shoots per original explant within 12 weeks at 4.0 mg L^{-1} BAP and maximum shoot length range (1-3 cm) after 90 days was also observed on same medium. Rooting (42%) of micropropagated shoots was achieved in half MS solid medium containing 2.5 mg L^{-1} IBA.

Key words: Jojoba, female plants, node, BAP, shoot multiplication.

Jojoba [*Simmondsia chinensis* (Link) Schneider] is an economically important dioecious plant native to Sonoran Desert of South Western USA and Northern Mexico. The dry seeds contain about 50% lipids in the form of simple wax esters having properties similar to those of sperm whale oil. The seed oil has been used in lubricants, cosmetics, pharmaceuticals and plastic industries. Jojoba may be propagated directly from seeds, but seeded plantation gives low average yields and 50% turn out to be male plants, while 8-10% are sufficient for pollination. As male plants are not only unproductive, but also suppress the development of adjacent females, rouging of superfluous males is imperative. To obtain proper female and male ratio [9:1] excess male plants identified only at time of flowering (3-4 years) need to be thinned out. Adopting tissue culture technique only female plants can be raised (Ghatnekar and Ghatnekar, 2000). *In vitro* plant regeneration has been described, but the multiplication rate was low (Mills *et al.*, 1997). The present study was thus planned to obtain plantlets at a quicker rate through formation of multiple shoots by using nodal stem segments from three mature female plants of jojoba.

Materials and Methods

Nodal segments with axillary buds from the tip of branches of three mature field grown female plants of jojoba with fully expanded leaves were used as an experimental material. These were washed in running tap water for 10-15 minutes and pretreated with 5% teepol (BDH) solution for 5-10 min. After 4-5 washings these were surface sterilized by dipping in 95% ethanol for 4-6 seconds and then immersed in 0.1% (w/v) $HgCl_2$ for 6-8 minutes and serially washed 3-4 times with sterilized double distilled water. For establishment of cultures, explants were inoculated on MS (Murashige and Skoog, 1962) medium supplemented with cytokinins and auxins (Table 1) or with different concentrations of BAP (1.0 to 5.0 mg L^{-1}) alone. Subculturing was done on the same medium after 25-30 days. After rooting of micropropagated shoots was attempted on half and full MS medium supplemented with growth hormones viz. IAA, IBA and NAA. Cultures were incubated at 25±1°C and exposed to 16 h photoperiod. Data were recorded after five weeks of incubation and analyzed statistically following two factorial CRD.

Results and Discussion

In one experiment random nodal segments excised from mature female plants were inoculated on MS medium fortified with different combinations of growth regulators (Table 1). On overall mean basis, it was found that female plant II gave maximum bud break percentage (57.16%). There was no significant difference between genotype

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I and III in this regard. MS medium supplemented with 1.0 mg L^{-1} BAP and 0.01 mg L^{-1} NAA was best giving highest bud break percentage (82.4%) followed by MS medium with 2.0 mg L^{-1} BAP and 0.01 mg L^{-1} NAA giving a mean response of 81.0% and responses were not significantly different among each other. MS medium fortified with 1 mg L^{-1} kinetin and 0.05 mg L^{-1} IAA gave minimum bud break percentage (27.6%). There was a gradual increase in percentage of bud break when concentration of kinetin was increased from 1.0 to 3.0 mg L^{-1} keeping concentration of IAA constant, similar trend was obtained with zeatin (1.0 to 3 mg L^{-1}) and NAA (0.25 mg L^{-1}). However, no shoot multiplication was obtained in this experiment.

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. Further the sex of the plantlets thus obtained cannot be known until flowering. Hence in the present studies we also used BAP alone and cultured nodal segments from mature female plants of jojoba.

In second experiment explants consisting of two nodes with apical bud of female plants of *S. chinensis* were used for *in vitro* bud proliferation using BAP concentrations ranging from 1.0 to 5.0 mg L^{-1}. It was observed that on overall mean basis, highest bud break percentage (70.33) was shown at 5.0 mg L^{-1} of BAP. There were significant differences between the three female genotypes with respect to bud break percentage (Table 2).

In third experiment first, second and third nodal explants from tip of branches of all the three female plants were cultured on MS medium supplemented with different concentrations of BAP ranging from 1.0 mg L^{-1} to 5.0 mg L^{-1} so as to choose best node and best BAP concentration for direct multiplication. Number of days required for bud break ranged from 7-13 with 5.0 mg L^{-1} of BAP while this range was 15-20 days with other concentrations of BAP. A gradual increase in axillary shoot bud proliferation/multiplication percentage was observed with increasing BAP concentration from 1.0 to 4.0 mg L^{-1}. In case of one female genotype (I) 33.30% (Table 3) bud proliferation was observed on MS medium having 4.0 mg L^{-1} BAP from second node. For 2nd and 3rd plants maximum values for bud proliferation were 83.30% (for first node of second genotype) and 50% (for second node of third genotype), respectively. Out of three tested female genotypes, only one (II) was found to be superior (33.53%) for bud proliferation/multiplication on overall mean basis. Elhag *et al.* (1998) cultured shoot tip explants from seven genotypes (4 female and 3 male) on MS, B5 basal media containing different

Table 1. In vitro response of nodal segments excised from three mature female plants of Simmondsia chinensis on MS medium supplemented with different growth regulators

Medium composition (mg L^{-1})	% bud break			Mean
	Plant I	Plant II	Plant III	
BAP (1.0) + NAA (0.001)	75.0 (60.0)	78.0 (62.0)	74.5 (59.6)	75.8 (60.5)
BAP (2.0) + NAA (0.01)	50.0 (45.0)	60.0 (50.8)	55.5 (48.1)	55.1 (47.9)
BAP (3.0) + NAA (0.02)	56.2 (48.6)	60.0 (50.8)	55.0 (49.8)	57.0 (49.1)
BAP (1.0) + NAA (0.01)	80.2 (63.6)	85.0 (67.4)	82.0 (64.9)	82.4 (65.2)
BAP (2.0) + NAA (0.01)	80.2 (63.6)	82.5 (65.3)	80.2 (63.5)	81.0 (64.1)
BAP (3.0) + NAA (0.01)	75.0 (60.0)	80.0 (63.5)	76.9 (61.2)	77.3 (61.5)
Kin (1.0) + IAA (0.05)	25.0 (30.0)	30.0 (33.2)	28.0 (31.9)	27.6 (31.6)
Kin (2.0) + IAA (0.05)	31.2 (33.9)	35.5 (36.5)	33.0 (35.0)	33.2 (35.1)
Kin (3.0) + IAA (0.05)	37.6 (37.8)	45.0 (42.1)	35.0 (36.2)	39.2 (38.7)
Zeatin (1.0) + NAA (0.25)	37.6 (37.8)	40.0 (39.2)	38.5 (38.3)	41.0 (39.9)
Zeatin (2.0) + NAA (0.25)	37.6 (37.8)	45.5 (42.4)	40.0 (39.2)	41.0 (39.9)
Zeatin (3.0) + NAA (0.25)	43.5 (41.2)	44.5 (41.8)	42.3 (40.5)	43.4 (41.2)
Mean	52.4 (46.4)	57.2 (49.1)	53.4 (46.9)	

CD 0.05; Genotype= 0.99; Media= 1.15; Different concentrations= NS; Figures in parenthesis indicate transformed values.

Table 2. In vitro bud proliferation from explants consisting of two nodes with apical bud from three female plants of Simmondsia chinensis on MS medium supplemented with different concentrations of BAP

Female genotypes	BAP (mg L^{-1})					
	1.0	2.0	3.0	4.0	5.0	Mean
I	45.0 (42.1)	58.0 (49.6)	60.0 (50.7)	65.0 (53.7)	68.0 (55.5)	59.2 (50.3)
II	60.0 (50.7)	70.0 (56.8)	75.0 (59.9)	88.0 (69.7)	85.0 (67.2)	75.6 (60.9)
III	45.0 (42.1)	52.3 (46.3)	50.0 (45.0)	56.0 (48.4)	58.0 (49.6)	52.2 (46.3)
Mean	50.0 (54.0)	60.1 (50.9)	61.6 (51.9)	69.6 (57.3)	70.3 (57.4)	

CD 0.05; Genotypes = 1.45; BAP concentration = 1.88; Plants x BAP concentration = 3.26;
Figures in parenthesis indicate transformed values.

combinations of IAA and BAP and their data revealed significant genotypic and growth regulator effect. Recently Tyagi and Prakash (2004) also observed differential hormonal requirements of N^6-benzyladenine (BA) for optimum shoot regeneration from nodal explants of different genotypes as well as sex. However, a maximum of ten shoots per female nodal explant was obtained.

In the present studies 5-14 shoots per original explant within 12 weeks were obtained on MS medium with 4.0 mg L^{-1} BAP. This number of shoot was considerably higher as compared to earlier reports using explants from mature elite plants of jojoba (Chaturvedi and Sharma, 1989; Mills *et al.*, 1997). Agrawal *et al.* (2002) cultured nodal explants of 18 to 20-year-old female plants of *S. chinensis* on MS, B5, Knop or WPM media with different growth regulators and obtained on an average of 2.7±0.4 shoots when explants were cultured on MS medium supplemented with 20 µM BA. Maximum shoot length range (1-3 cm) after 90 days was also observed on MS medium with BAP 5.0 mg L^{-1} in present studies. Roussos *et al.* (1999) also observed an average shoot length = 2.3 cm on MS medium with 4.0 mg L^{-1} BAP. Khanam *et al.* (1999) also observed that culture medium supplemented with 4.0 mg L^{-1} of BAP was better for micropropagation of jojoba using female axillary bud explants as experimental material.

Table 3. Bud proliferation in nodal explants from three female plants of Simmondsia chinensis cultured on MS medium supplemented with different concentrations of BAP

Female genotypes	Explants	Concentration of BAP (mg L^{-1})					
		1.0	2.0	3.0	4.0	5.0	Mean
I	Node 1	8.3 (16.7)	8.3 (16.7)	12.5 (20.6)	12.5 (20.6)	8.3 (16.7)	9.9 (18.3)
	Node 2	16.6 (24.0)	16.6 (24.0)	25.0 (29.9)	33.3 (35.2)	25.0 (29.9)	23.3 (28.6)
	Node 3	8.3 (16.7)	8.3 (16.7)	16.6 (24.0)	20.8 (27.1)	8.3 (16.7)	20.7 (20.2)
	Mean	11.0 (19.1)	11.0 (19.1)	18.0 (24.9)	22.2 (27.6)	13.8 (21.1)	17.9 (22.4)
II	Node 1	12.5 (20.6)	12.5 (20.6)	16.6 (24.0)	83.3 (65.8)	16.6 (24.0)	28.3 (31.0)
	Node 2	16.6 (24.0)	33.3 (35.2)	40.0 (39.2)	60.0 (50.7)	60.0 (50.7)	41.9 (39.9)
	Node 3	18.3 (25.3)	40.0 (39.2)	18.3 (25.3)	50.0 (44.9)	25.0 (29.9)	30.3 (32.9)
	Mean	15.8 (23.3)	28.6 (31.7)	24.9 (29.5)	64.4 (53.8)	33.8 (34.9)	33.5 (34.6)
III	Node 1	4.1 (11.7)	4.1 (11.7)	8.3 (16.7)	16.6 (24.0)	8.3 (16.7)	8.3 (16.2)
	Node 2	8.3 (16.7)	40.0 (39.2)	33.3 (35.2)	50.0 (44.9)	33.3 (35.2)	32.9 (34.2)
	Node 3	8.3 (16.7)	33.3 (35.2)	40.0 (39.2)	50.0 (44.9)	33.3 (35.2)	32.9 (34.2)
	Mean	6.9 (15.0)	25.8 (28.7)	27.2 (30.3)	38.8 (37.9)	24.9 (29.0)	24.7 (28.2)
Overall mean		11.2 (19.1)	21.8 (26.5)	23.4 (28.2)	41.8 (39.8)	24.3 (28.3)	

Overall mean for nodes: Node 1 = 15.52; Node 2 = 32.75; Node 3 = 28.00
CD (P = 0.05)

Genotypes	= 0.19	Genotype x Node	= 0.34
Node	= 0.19	Genotype x BAP concentration	= 0.43
BAP concentration	= 0.25	Node x BAP concentration	= 0.43
		Genotype x Node x BAP concentration	= 0.76;

Figures in parenthesis indicate transformed values.

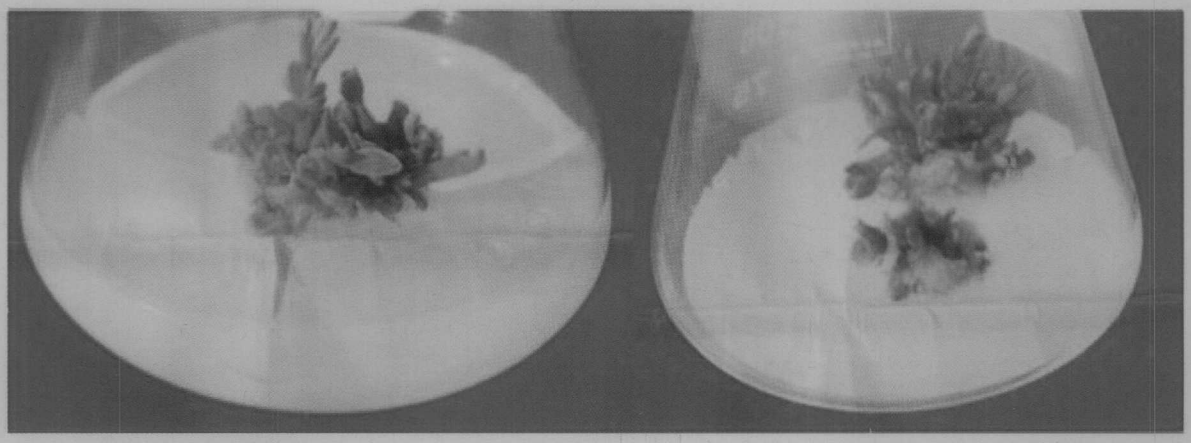

Fig. 1. Multiple shoot formation from second and third nodes of Simmondsia chinensis on MS medium supplemented with 4.0 mg L^{-1} BAP.

Among all three nodes tested, on overall mean basis, second node (32.7%) and third node (28%) (Fig. 1) gave good response for shoot multiplication as compared to the first node (15.52%). However, percentage of bud break was high in explants consisting of two nodes with apical bud as compared to 1st, 2nd, or 3rd nodal segments, but multiplication rate was high in the latter case. Shoots were transferred onto full strength MS solid medium or supplemented with 10 mg L^{-1} each of IBA and NAA or half MS liquid medium having IAA or IBA 2.5 mg L^{-1} each and then after two days, these were transferred to half MS solid medium, but there was no rooting. Rooting (25%-42%) of micropropagated shoots was observed on half strength MS basal solid medium supplemented with 1.0 mg L^{-1} or 2.5 mg L^{-1} IBA. However, it took 45-50 days for the appearance of roots. The number of roots per shoot ranged between 3 and 11. However, Mills *et al.* (1997) reported a requirement of 120 mg L^{-1} of IBA for inducing 80% rooting. Chaturvedi and Sharma (1989) reported that a combination of 1 mg L^{-1} NAA and 7 mg L^{-1} IBA was more effective for rooting than their application alone. Three to four roots developed in each shoot along with a significant mass of callus. According to them this type of root system did not facilitate good acclimatization upon transplanting into soil.

In the present studies attempts were also made to obtain plantlets through callus cultures raised from leaf, internode and node explants. The calli were transferred to MS medium containing different growth regulators. However, the only positive response towards regeneration was greening of calli. The formation of green calli indicated that the callus cells have the capacity to differentiate, but for want of proper impulse in the form of growth regulators or other adjuvants the cells were not able to express their potentiality.

Jojoba, evergreen bushy shrub, has emerged as a promising crop for cultivation in wastelands and arid zones throughout the world. MS basal medium fortified with 4 mg L^{-1} of BAP was found to be ideal for shoot proliferation of jojoba. A good rooting response in terms of number of roots was obtained in medium with 2.5 mg L^{-1} IBA. As the number of roots is more significant than their length for survival during the hardening stage (Gill *et al.*, 1994), IBA alone was more effective and 3-11 roots per shoot could be induced. This number of roots seems to be quite sufficient for successful transplantation of the plantlets to the natural conditions. Bud proliferation/ multiplication along with root differentiation from mature female jojoba plants can be of great help for *in vitro* propagation of this important and underutilized plant species.

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