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In Vitro Propagation of Jojoba (*Simmondsia chinensis* L.) through Alginate-encapsulated Shoot Apical and Axillary Buds

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ABSTRACT

Regrowth of apical and axillary buds of jojoba after encapsulation in calcium alginate matrix was evaluated. These buds were derived from *in vitro* cultured shoots. Encapsulation was performed with 6% sodium alginate and 100 mM $CaCl_2$. Morphogenic response and percentage of conversion into plantlets of encapsulated buds on various planting media was described. Encapsulated buds exhibited the best shoot development on Murashige and Skoog (MS) medium supplemented with 1.0 mg/L BAP, 40mg/L adenine sulfate and 3.0 mg/l IAA and gelled with 0.8% bacteriological agar (73% $\pm$5.17 conversion). This was followed by hormone-free MS basal medium (61% $\pm$4.9). Culturing encapsulated buds on peat moss: sand (2:1) moistened with MS liquid medium had the lowest conversion percentage (48% $\pm$3.62). Plantlets regenerated from the encapsulated buds were hardened-off and transferred to soil.

Key Words: *in vitro* propagation; Calcium alginate; Encapsulation; *Simmondsia chinensis*; Jojoba

INTRODUCTION

Jojoba {*Simmondsia chinensis* (Link) Schneider} is an economically important dioecious plant native to Sonoran desert of South Western USA and Northern Mexico (Hogan, 1979). The seeds of jojoba produce a liquid wax that can be used as a substitute for sperm whale oil. The wax is of immense commercial value, particularly as a high-pressure lubricant (Yermanos, 1979). Jojoba oil is also used in cosmetic industry, hair care products, in pharmaceuticals and also as low calories edible oil (Naqvi & Ting, 1990). The vegetative propagation of this plant by usual horticultural methods is difficult. Rooting of cuttings, besides being slow, requires considerable controlled greenhouse facilities. Furthermore, there are certain other horticultural limitations, since from a particular elite individual only a few cuttings can be obtained and the rooting can be achieved only if the cuttings of newly hardened terminal shoots are taken during a particular period of the year (Chaturvedi & Sharma, 1989).

Plant tissue culture technology has a potential to overcome these problems. Micropropagation is one of the most worldwide methods of biotechnology, allows efficient and rapid clonal propagation of many economically important crops. However, the low percent survival of *in vitro* plantlets during the *ex vitro* acclimatization stage is a real problem. Efforts have been intensified in overcoming this problem by encapsulating somatic embryos and shoot bud explants in different matrices and attempting to grow these synthetic seeds (Synseeds) on different substrata (Aitken-Christie *et al*., 1995).

According to Bornman (1993) synthetic seeds may provide the only technology realistically amenable to the extensive scale up required for the commercial production of some clones. In addition, Mathur *et al*. (1989) reported that the use of this technology economized upon the medium, space and time requirements.

Successful cases of synthetic seeds production and plantlet regeneration have been reported for cereals, vegetables, fruits, ornamentals, aromatic grass and conifers (Fowke *et al*., 1994; Piccioni & Standardi, 1995; Janeiro *et al*., 1997; Castillo *et al*., 1998; Mamiya & Sakamoto, 2001; Ganapathi *et al*., 2001; Brischia *et al*., 2002; Hao & Deng, 2003). However, in most cases somatic embryos were used in the encapsulation process. Few reporters (Mathur *et al*., 1989; Ganapathi *et al*., 1992; Shamara *et al*., 1994; Piccioni & Standardi, 1995; Pattnaik & Chand, 2000) described the encapsulation of vegetative propagules such as axillary buds or shoot tips which could be used for mass clonal propagation as well as in long-term conservation of germplasm. The present study described the encapsulation of shoot tips and axillary buds obtained from *in vitro* shoot culture of jojoba (*S. chinensis* L.) in calcium alginate gel, and the successful development of plantlets from these capsules on various planting media.

MATERIALS AND METHODS

Plant material. Seeds of jojoba were obtained from the Egyptian Company for Natural Oils, Cairo, Egypt. Seeds were surface sterilized with 0.1% mercuric chloride for 10 minutes, washed four times with sterilized distilled water. Seeds were de-coated and germinated under aseptic conditions on MS (Murashige & Skoog, 1962) basal medium supplemented with 1.0 mg/L BAP. Proliferated shoots were collected and apical and axillary buds were removed by cutting the shoot axis just above and below the nodes. Apical buds and shoot segments, approximately 3 mm long; each including one node with an axillary bud; were used as explants for encapsulation. No distinction

between apical and axillary buds was made as all explants were randomly mixed and used as experimental units.

Encapsulation matrix. Different concentrations (2, 4, 6 and 8%) of sodium alginate (Duchefa Biochemie BV, Netherlands) were prepared using MS liquid medium supplemented with 3% sucrose, 40 mg L^{-1} adenine hemi-sulfate, 1 mg L^{-1} BAP and 3 mg L^{-1} IAA. For complexation 25, 50, 75 and 100 mM $CaCl_2$ solution was prepared. Both sodium alginate and $CaCl_2$ were autoclaved at 121ºC for 15 minutes after adjusting the pH to 5.8 $\pm$ 0.1.

Encapsulation, planting media and culture conditions. Sodium alginate was pipetted with a wide mouth pipette into the bottom of sterilized petri-dish containing $CaCl_2$ solution. Explants were immersed with a pair of forceps into the sodium alginate as it falls into the $CaCl_2$. Calcium alginate capsules containing buds were collected after one hour and rinsed 3 times with sterilized distilled water to wash away calcium chloride residues.

Encapsulated buds were planted onto petri-dishes containing the following media: hormone-free MS basal medium gelled with 0.8% bacteriological agar (M_1); MS medium supplemented with 1 mg L^{-1} BAP, 40 mg L^{-1} adenine sulfate, 3 mg L^{-1} IAA and gelled with 0.8% agar (M_2); Peat-moss: sand (2:1) moistened with half-strength MS liquid medium (M_3) and Peat-moss: sand (2:1) moistened with tap water (M_4).

All media were sterilized before use by autoclaving for 15 minutes at 121ºC. All operations were carried out under aseptic conditions. Cultures were maintained at 28ºC$\pm$1 under 16 hours photoperiod. Each medium was represented by 6 petri-dishes each containing 10 capsules as replicates. Six weeks later, the converted plantlets developed on each media were counted and transferred to small pots containing peat-moss: sand (2:1) for hardening off before field planting.

Statistical analysis. Student's t. Test was used for the comparison of different means.

RESULTS AND DISCUSSION

The formation of fir, uniform calcium alginate capsules was achieved with 6% sodium alginate complexed with 100 mM $CaCl_2$. Lower concentrations of sodium alginate or $CaCl_2$ prolonged the time of ion exchange of sodium and calcium ions and resulted in fragile capsules. Using 8% sodium alginate solution produced very hard capsules. It seems that the source of the alginate affects the quality of the capsules. Pattnaik and Chand (2000) reported that the complexing ability of the sodium alginate (commercial source: CDH and GSS, India) varied considerably with the source. They found that 4% of the former produced firm clear, uniform capsules while using alginate from the later failed to complex with $CaCl_2$ at all concentrations tested. Castillo *et al.* (1998) formed good beads of uniform size and shape with 2.5% sodium alginate (source: Sigma and Fluka). Mathur *et al.* (1989) reported that 6% solution of sodium alginate (source: Kothari Phytochemical, India) upon complexation with 75 mM $CaCl_2.2H_2O$ solution gave the optimal, firm and round beads.

Table I. Effect of different media on conversion of encapsulated apical and axillary buds into plantlets of jojoba after five weeks. M_1: hormone-free MS basal medium jelled with 0.8% agar; M_2: Ms medium supplemented with 1.0 mg/L BAP, 40 mg adenine sulfate, 3.0 mg/LIAA and gelled with 0.8% agar; M_3:peat-moss: sand (2:1) moistened with ½ MS liquid medium: M_4: peat-moss: sand (2:1) moistened with tap water

Media	Shoot length (cm) $\pm$ SE	Root length (cm) $\pm$ SE	% Conversion $\pm$ SE
M_1	2.6 + 0.76	1.5 + 0.30	61 + 4.91
M_2	3.4 + 0.90	1.8 + 0.89	73 + 5.17
M_3	2.1 + 0.17	1.1 + 0.34	48 + 3.62
M_4	0.8 + 0.13	-----	10 + 1.78

In the present study, buds encapsulated in 6% calcium alginate and cultured on three of the four different media, described above, showed the emergence of shoots and roots two to three weeks after incubation. The frequency of plantlets development on these culture media varied according to medium composition (Table I). MS medium supplemented with 1mg L^{-1} BAP, 40 mg L^{-1} adenine sulfate, 3 mg L^{-1} IAA and (M_2) gave the maximum frequency of conversion of encapsulated buds into plantlets (73% $\pm$5.17, Fig. 1a). After five weeks, a well developed shoots and roots were observed on this medium. The average length of the shoots was 3.4 cm$\pm$0.9 and that of the roots was 1.8 cm $\pm$0.89 (Table I, Fig. 2a). The frequency of conversion of culturing encapsulated buds on hormone-free MS basal medium (M_1) was 61%$\pm$4.91. The average length of the shoots was 2.6 cm$\pm$0.76 and that of the roots was 1.5 cm$\pm$0.3 (Table I). The frequency of conversion on M_3 was 48%$\pm$3.62 (Fig. 1b) with average shoot and root length 2.1cm$\pm$0.17 and 1.1$\pm$0.34, respectively. Encapsulated explants cultured on M_4 showed the emergence of weak shoots (0.8 cm$\pm$0.13) with no roots, which failed to continue growth and died after yellowing. Similar results have been obtained by Ganapathi *et al.* (2001) who encapsulated somatic embryos of banana and cultured them on different media (MS supplemented with different growth regulators) and substrates (cotton, soilrite and blotting paper) moistened with ¼ strength liquid MS medium. They reported that the frequency of conversion on these substrates (20% on cotton and soilrite) was much lower than on nutrient MS medium (66%). Bapat and Rao (1990) under non-aseptic conditions, encapsulated axillary buds of mulberry in autoclaved alginate matrix containing fungicides and sown them directly in soil irrigated every other day with half-strength MS medium without sucrose. They also grew encapsulated buds on cotton or filter paper and reported that with soil,

Fig. 1. Extrusion of shoots and green leaves from calcium alginate capsules, after 3 weeks of culture, grown on (a) MS basal medium supplemented with 1.0 mg/L BAP, 40 mg/L adenine sulfate and 3.0 mg/L IAA (M_2) (X 1.0) and (b) Peat-moss: sand (2:1) moistened with ½ MS liquid medium (M_3) (X 1.0)

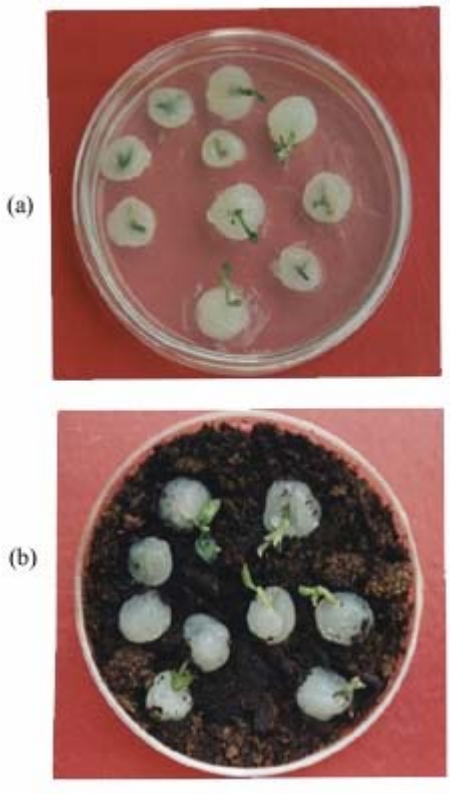

Fig. 2. a- A well defined shoot and root derived from a calcium alginate capsule grown on M_2 after five weeks of culture (X 1.5), b- two month-old hardened jojoba plantlets derived from encapsulated buds grown on M_2 (X 0.6

60% of the beads showed shoot emergence while it was 20% with cotton and 15% with filter paper.

In this study, the best conversion frequency into plantlets on using medium M_2, could be attributed to the inclusion of MS salts and hormones into the encapsulation matrix. Ganapathi *et al.* (2001) suggested that ingredients in the encapsulation matrix contribute like an artificial endosperm to the developing encapsulated somatic embryos of banana. Antonietta *et al.* (1999) reported that a synthetic endosperm should contain nutrients and carbon source for optimum germination and conversion. In addition, Rao and Singh (1991) reported that the use of full strength MS medium gave the highest conversion frequency in *Solanum melongena*. However, half-strength MS medium was found to be effective for the germination of encapsulated somatic embryos of papaya (Castillo *et al*., 1998).

Mathur *et al*. (1989) reported that the highest plant formation frequency (96-100%) of encapsulated buds of *Valeriana wallichii* was observed on 0.7% agar, both with or without MS nutrients, while non-encapsulated buds under the same conditions failed to grow and died following yellowing.

In the present study, plants regenerated from the encapsulated apical and axillary buds of jojoba (*Simmondsia chinensis* L.) were transferred after hardening off (Fig. 2b) to field conditions. All regenerated plants showed no morphological variations when compared with the original mother plant.

CONCLUSION

The study indicated that it is possible to use the apical and axillary buds of jojoba for encapsulation and that this technology was successful for clonal propagation of such economically important plant.

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