Alginate-encapsulation of shoot tips of jojoba [*Simmondsia chinensis* (Link) Schneider] for germplasm exchange and distribution

Sunil Kumar · Manoj K. Rai · Narender Singh ·
Manisha Mangal

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Abstract Shoot tips excised from *in vitro* proliferated shoots derived from nodal explants of jojoba [*Simmondsia chinensis* (Link) Schneider] were encapsulated in calcium alginate beads for germplasm exchange and distribution. A gelling matrix of 3 % sodium alginate and 100 mM calcium chloride was found most suitable for formation of ideal calcium alginate beads. Best response for shoot sprouting from encapsulated shoot tips was recorded on 0.8 % agar-solidified full-strength MS medium. Rooting was induced upon transfer of sprouted shoots to 0.8 % agar-solidified MS medium containing 1 mg l⁻¹ IBA. About 70 % of encapsulated shoot tips were rooted and converted into plantlets. Plants regenerated from encapsulated shoot tips were acclimatized successfully. The present encapsulation approach could also be applied as an alternative method of propagation of desirable elite genotype of jojoba.

Keywords Acclimatization · Conservation · Plant regeneration · *Simmondsia chinensis* · Synthetic seeds

Introduction

Encapsulation technology has received considerable attention as a potentially cost-effective propagation system (Rai et al. 2009). Successful cases of synthetic seed production

and plantlet regeneration have been reported for a wide range of plants including cereals, vegetables, fruits, ornamentals, medicinal plants and forest trees (Redenbaugh et al. 1991; Castillo et al. 1998; Ara et al. 2000; Mandal et al. 2000; Rai et al. 2009). In most cases somatic embryos have been used in the encapsulation process, however, in recent years adequate efforts have also been made to explore the possibility of encapsulating non-embryogenic, *in vitro*-derived vegetative propagules such as axillary buds, shoot tips or nodal segments for synthetic seed production (Mathur et al. 1989; Ganapathi et al. 1992; Sharma et al. 1994; Ara et al. 2000; Mandal et al. 2000; Rout et al. 2001; Chand and Singh 2004; Naik and Chand 2006; Singh et al. 2006a, b, 2009, 2010; Faisal and Anis 2007; Micheli et al. 2007; Rai et al. 2008a, b, 2009; Lata et al. 2009; Sundararaj et al. 2010; Verma et al. 2010). Encapsulation of vegetative propagules could be used for mass propagation of high value crops at a reasonable cost especially when somatic embryos are not available for encapsulation. In addition, these propagules could also be useful in conservation of germplasm of elite and endangered plants or to facilitate exchange of axenic materials between laboratories and Extension centres (Rai et al. 2009).

Jojoba (*Simmondsia chinensis*), which belongs to the family Simmondsiaceae, is an evergreen, dioecious desert shrub which grows wild in Sonora desert of Arizona, northern Mexico, southern and Baja California (Kumar et al. 2009). Jojoba is now cultivated commercially in Argentina, Australia, Chile, Egypt, India, Israel, Mexico, Peru, South Africa and USA. Jojoba seed oil is being utilized in industrial lubricants, and in pharmaceutical and cosmetic industries. The liquid wax that makes 40–60 % of seed dry weight has properties similar to sperm whale oil (Wang and Janick 1986). Due to the ban on the import of sperm whale products into USA and other countries, jojoba is gaining commercial

S. Kumar (✉) · M. K. Rai · M. Mangal
Centre for Plant Biotechnology, CCS Haryana Agricultural
University Campus,
Hisar 125 004, India
e-mail: sunyad84@gmail.com

S. Kumar · N. Singh
Department of Botany, Kurukshetra University,
Kurukshetra 136 119, India

importance at international level. Therefore, it is necessary to develop an efficient method of transport of tissue culture raised and uncontaminated healthy plants which can fulfill the demands of industry. In this context, encapsulation technology could be useful for the exchange of germplasm of elite genotypes and axenic plant material between laboratories and pharmaceutical industries due to the small bead size and relative ease of handling of these structures (Danso and Ford-Lloyd 2003; Naik and Chand 2006; Rai et al. 2009; Singh et al. 2010). Hence, this investigation is aimed to develop an efficient method of germplasm exchange and distribution through synthetic seeds in *S. chinensis*.

Materials and methods

Nodal segments from field grown mature plants of *S. chinensis* were used to initiate *in vitro* cultures. Explants were washed thoroughly for 30 min under running tap water to remove the dust particles, and surface sterilized with 70 % ethanol for 30 s followed by 0.1 % mercuric chloride for 3–4 min, and rinsed 4–5 times with sterile distilled water under aseptic condition. For shoot regeneration, nodal segments were cultured on MS (Murashige and Skoog 1962) medium supplemented with 2.0 mg l⁻¹ 6-benzylaminopurine (BAP) and 0.5 mg l⁻¹ α -naphthaleneacetic acid (NAA). The pH of the medium was adjusted to 5.8 $\pm$ 0.02 using 0.1 N NaOH and 0.1 N HCl prior to adding 0.8 % agar-agar (Hi-media, India). The medium was autoclaved at 121 °C at 1.1 kg cm⁻² for

15 min. Cultures were maintained at 24 $\pm$ 2 °C with a 16 h photoperiod at a photon flux density (PFD) of 40 μ mol m⁻² s⁻¹ from cool white fluorescent tubes (Philips, India). Shoot tips (3–5 mm) excised from *in vitro* proliferated shoots (Fig. 1a), obtained from nodal segments were used as explants for synthetic seed production in *S. chinensis*.

For the encapsulation, sodium alginate (Hi-Media, India) at 2–5 % (w/v) was used as the gelling agent whereas 100 mM calcium chloride (Hi-Media, India) was used as the complexing agent. Aliquots of the alginate solution, each of which contained one shoot tip, were taken up with a sterile spatula and then gently dropped into 100 mM calcium chloride solution. The droplets containing shoot tips were held for 20–30 min in the calcium chloride solution to achieve polymerization of sodium alginate. After hardening, the alginate beads (6–7 mm) were washed 3–4 times with double-distilled water. In order to recover plantlets under *in vitro* conditions, encapsulated shoot tips were cultured in following growth media: (i) M₁, 0.8 % agar-solidified full-strength MS medium (ii) M₂, liquid full-strength MS medium and (iii) M₃, 0.8 % agar-solidified MS medium containing 1 mg l⁻¹ BAP. To evaluate the effect of medium strength on shoot regeneration, encapsulated shoot tips were cultured on 0.8 % agar-solidified full, half or quarter strength MS medium. For rooting, shoots emerged from encapsulated shoot tips on MS medium were transferred to 0.8 % agar-solidified full-strength medium containing 1 mg l⁻¹ indole-3-butyric acid (IBA). All cultures were maintained as previously described.

Fig. 1 Plant regeneration of *S. chinensis* from encapsulated shoot tips. **a.** Multiple shoots induction from nodal explants. **b.** Shoot tips encapsulated in calcium alginate beads. **c.** Shoot emergence from encapsulated shoot tips. **d.** Shoot (s) and root (r) emergence from encapsulated shoot tip. **e.** Hardened plants of *S. chinensis*

Plantlets (3–4 cm) with well developed shoot and roots, regenerated from encapsulated shoot tips, were thoroughly washed in running tap water by using fine brush and were transferred to small pots containing sterilized sand. These pots were supplied with half-strength MS salt solution and covered with polythene bags to maintain high relative humidity. These were placed in growth chamber at 25 ± 2 °C under 16 h photoperiod. After 2 weeks, polythene bags were removed gradually ($3\text{--}4$ h d^{-1}) for their acclimatization to natural humidity and sun light. After 6 weeks plantlets were transferred along with sand to the pots filled with garden soil and kept in greenhouse for the normal growth.

For the above experiments, 24 explants were used for each treatment and each experiment was repeated thrice. The results are expressed as a mean $\pm$ SE of three independent experiments. The data were analyzed statistically using one-way analysis of variance (ANOVA) and the significant differences between means were assessed by Duncan's multiple range test at $P < 0.05$.

Results and discussion

In the present investigation, shoot tips excised from *in vitro* proliferated shoots were used as an explant for the development of synthetic seed in *S. chinensis*. Shoot tips generally yield better response than other non-embryogenic vegetative propagules. The reason for such differences in response may be due to greater mitotic activity in shoot tips than in lateral buds, which are subjected to apical dominance (Verma et al. 2010). Recently, micropropagation through encapsulated shoot tips has also been reported in several economically important plants (Singh et al. 2006a; b; 2009; Faisal and Anis 2007; Rai et al. 2008a; Ray and Bhattacharya 2008; Verma et al. 2010). Critical evaluation of concentrations of sodium alginate and calcium chloride which affects the gel matrix and capsule quality is one of the important aspects for the successful propagation of plants through encapsulation technology. Capsule hardness depends upon optimal ion exchange of Na^+ and Ca^{2+} and it may vary with different propagules as well as with different plant species (Singh et al. 2009; Rai et al. 2009). In the present study, the presence of 3 % sodium alginate and 100 mM calcium chloride was found best composition for gel complexation, which produces firm, clear, and isodiametric beads (Fig. 1b). Lower concentrations of sodium alginate (1–2 %) not only prolonged the polymerization duration, but also resulted in fragile beads, which were difficult to handle, whereas at higher concentrations of sodium alginate (4–5 %), beads were too hard causing considerable delay in shoot emergence.

Table 1 Shoot regeneration from encapsulated shoot tips on different growth media

Medium Composition	Response for shoot sprouting (%)	Shoot length (cm)
M ₁	66.6 $\pm$ 2.3 a	2.26 $\pm$ 0.12 a
M ₂	49.9 $\pm$ 4.8 b	1.33 $\pm$ 0.08 b
M ₃	29.1 $\pm$ 2.4 c	0.92 $\pm$ 0.05 c
LSD ($P < 0.05$)	11.8	0.32
ANOVA	$F_{2,6} = 30.4^{**}$	$F_{2,6} = 57.26^{**}$

M₁, 0.8 % agar-solidified full-strength MS medium; M₂, full-strength liquid MS medium M₃, 0.8 % agar-solidified full-strength MS medium + 1 mg l^{-1} BAP

Values are means $\pm$ S.E. of three independent experiments. Data were recorded after 4 weeks of culture. Mean values sharing the same letter do not differ significantly ($P < 0.05$) according to Duncan's multiple range test

** Significant at $P < 0.01$

Irrespective of media composition, encapsulated shoot tips exhibited shoot development after 2–3 weeks of culture (Fig. 1c). Of the three different medium composition tested, best response for shoot sprouting from encapsulated shoot tips was recorded on M₁ medium (Table 1). However, contrary to some previous reports (Rai et al. 2008a; Singh et al. 2009; Verma et al. 2010), all three medium composition inhibited the rooting in shoots that emerged from encapsulated shoot tips, which was overcome after transfer of sprouted shoots to 0.8 % agar-solidified MS medium containing 1 mg l^{-1} IBA after 4 weeks. After transfer to IBA containing medium, about 70 % of encapsulated shoot tips were rooted (Fig. 1d) and converted into plantlets. The major limiting factor for one-step germination of encapsulated vegetative propagules is their unipolar nature. Unlike somatic embryos, these vegetative

Table 2 The effect of different strength of MS medium on shoot regeneration from encapsulated shoot tips

Medium strength	Response for shoot sprouting (%)	Shoot length (cm)
Full	66.6 $\pm$ 2.3 a	2.26 $\pm$ 0.12 a
Half	40.2 $\pm$ 3.6 b	1.03 $\pm$ 0.03 b
Quarter	22.2 $\pm$ 2.8 c	0.90 $\pm$ 0.05 b
LSD ($P < 0.05$)	10.35	0.27
ANOVA	$F_{2,6} = 55.3^{**}$	$F_{2,6} = 90.17^{**}$

Medium: 0.8 % agar-solidified MS medium

Values are means $\pm$ S.E. of three independent experiments. Data were recorded after four weeks of culture. Mean values sharing the same letter do not differ significantly ($P < 0.05$) according to Duncan's multiple range test

** Significant at $P < 0.01$

propagules are unipolar, which do not have a root meristem, hence, an appropriate root induction treatment needs to be incorporated with encapsulation protocol (Naik and Chand 2006; Rai et al. 2008b).

Analysis of variance of data revealed that strength of MS medium had a significant ($P<0.05$) effect on sprouting and growth of shoots emerged from encapsulated shoot tips. Best results were recorded on full-strength medium in comparison to half or quarter strength medium (Table 2). This difference in response may be due to higher requirement of MS salts and vitamins. Similar observations were also made in banana (Ganapathi et al. 2001), *Phyllanthus amarus* (Singh et al. 2006a), guava (Rai et al. 2008a).

Well developed rooted plantlets regenerated from encapsulated shoot tips were hardened and approximately 60 % of the transferred plantlets became established in soil (Fig. 1e).

In conclusion, the present encapsulation approach will have a considerable impact on transportation of the germplasm and conservation of *S. chinensis* and could also be applied as an alternative method of propagation. Future studies will focus on the conservation of encapsulated shoot tips of jojoba for longer period using slow-growth procedures.

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