Clonal Propagation and *In Vitro* Conservation of Jojoba [*Simmondsia chinensis* (Link) Schneider]

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Simmondsia chinensis (Link) Schneider, popularly known as 'jojoba' or 'hohoba', is an evergreen, dioecious desert shrub, belonging to the family Simmondsiaceae. The plant is valued for its seeds, which store liquid wax (50% dry wt.). The properties of jojoba oil are similar to that of spermaceti and it is widely used as lubricant for heavy machinery, in cosmetic, pharmaceuticals and plastic industries (Benzioni, 1995). The vegetative propagation of jojoba *via* conventional stem cuttings did not prove effective due to lengthy procedure and slow growth (Lee *et al.*, 1985; Benzioni, 1995). Therefore, vegetative propagation of jojoba through tissue culture-raised plants becomes the obvious choice. The conservation of germplasm is imperative for the breeding programmes, as the diversity in the crop harbours genes for various important traits. Amongst the various approaches for *ex situ* conservation, tissue culture is potential technique for conservation of those plants where either seeds are not set or heterozygosity is prevalent among seeds. *S. chinensis* is dioecious in nature and cross-pollinated (Gentry, 1958). Therefore, *in vitro* conservation is the best alternative to maintain the true-to-type plants for such crops. Development of micropropagation protocol is a pre-requisite for *in vitro* conservation. Though some attempts have been made earlier to obtain the plants of jojoba clonally from sexually known plants using tissue culture (Chaturvedi and Sharma, 1989; Llorente and Apostolo, 1998) but none of these reports mention the differential responses of male and female explants. The present study aimed to investigate the twin objectives (a) clonal propagation *via* tissue culture and (b) *in vitro* conservation of male and female accession of *S. chinensis*.

Healthy twigs (20-30 cm long) were excised from 20-year-old female and male plants of *S. chinensis* (EC 99691 Ex-USA) growing at NBPGR Regional Research Station, Jodhpur, Rajasthan. The twigs were thoroughly washed under running tap water for 30 min. After defoliating, 20 nodal segments (1-2 cm) from each male and female plants were excised, surface sterilized with freshly prepared 0.2% $HgCl_2$ for 10 min and finally rinsed 4 or 5 times with sterile distilled water in laminar flow cabinet. The cut ends of the explants which had turned brown were trimmed prior to culture for their establishment in the medium. The pH of the media was adjusted to 5.8 with 1N HCl or 1N NaOH. The culture vessels (25 x 150 cm; Borosil) containing media (20 ml) enclosed with polypropylene caps were autoclaved at 121°C and 15 psi pressure for 15 min. All the cultures were incubated in a culture room at 25 ± 2°C under photo period 16 h provided by cool white fluorescent tubes (40 W, Philips, India) with 40 mmol/m²/s illumination. Further experiments were carried out using the nodal segments (*ca.* 1 cm) excised from the shoots obtained from mature explants in cultures. Murashige and Skoog's (hereafterward referred to as MS) medium (1962) was used as basal medium and served as control. The nodal explants were cultured on MS supplemented with various concentrations (2, 5, 10 µM) of N^6-benzyladenine (BA). The *in vitro* regenerated shoots were given a pulse treatment of 50 µM indole-3-butryic acid (IBA) for 1-20 min. and implanted on semi-solid MS + 10 µM IBA + 0.5% activated charcoal (AC) for induction of roots. Shoots with small roots; more aptly plantlets, were transferred to MS liquid medium (devoid of iron) supplemented with 5 µM BA and containing cotton as support matrix in the culture tubes for elongation. Shoot cultures obtained on control and MS + 2-10 µM BA were maintained as such (without subculture) and studied for conservation on their respective culture media.

Data were recorded for number of shoots/culture, shoot length (cm), % of rooting cultures, number of roots/shoot and root length (cm) after a periodic interval of 30 days. Conservation period was computed when the 50% shoots of a culture died and the remaining shoots were further subcultured on defined media. All the experiments replicated thrice; each replicate comprised 10 cultures. Infected cultures are not counted for computing the mean values. Therefore, data are based on 25-30 cultures for each treatment.

Following the surface sterilization described earlier, 20% in male and 52% in female explants established in cultures. Among the various concentrations of cytokinin tested, 10 µM BA supported shoot regeneration in nodal explants excised from both male and female plants that were used for initial establishment of cultures. Two or three shoots/explant regenerated in 10% of male and 25% of female explants during 3 months on MS + 10 µM BA. For further experiments, the nodal explants derived from these shoots were used. Direct shoot regeneration occurred on all the media tested in cultures of both types of explants (male and female). Within 1 week, the shoot (0.3-0.5 cm long) regeneration was observed in all the cultures. Male nodal explants elicited the best response *i.e.* 4.1 shoots/culture, as obtained on MS + 5 µM BA in 60 d from culture. In 90-d-old cultures, the shoot propensity increased to 5.5 shoots/culture with 2.0 cm length on the same medium (Table 1). In female nodal explants, MS + 10 µM BA was found optimal for shoot regeneration (6.7 shoots/culture) in 60 days and subsequently 8.7 shoots/culture with 1.7 cm length were obtained in 90 d from culture on the same medium (Table 1). Direct shoot regeneration was obtained from axils of node of the explants, however, callus formation was observed from the basal cut ends. The effectiveness of BA for micropropagation of jojoba has already been documented (Llorente and Apostolo, 1998; Roussos *et al.*, 1999). However, Chaturvedi and Sharma (1989) reported 5-8 shoots/nodal explants of jojoba on Schenk and Hildebrandt (1972) supplemented with BA in combination with indole-3-acetic acid (IAA).

The shoots (1-2 cm) were excised and initially given a pulse treatment of 50 µM IBA for different duration (1-20 min) for *in vitro* rhizogenesis. The pulse treated-shoots were vertically implanted on MS + 10 µM IBA + 0.5% AC. For both the male and female shoots 50 µM IBA for 20 min proved to be the best as 53% of male shoots and 67% of female shoots developed roots within 30 d (Table 2). To facilitate elongation of roots, the shoots (1-2 cm) with small roots (2-3 cm) were transferred to MS liquid medium (devoid of iron) containing 5 µM BA and cotton as support matrix. Shoots as well as roots elongated to 4-5 cm each in 2 months on the same medium. The plantlets are ready for hardening treatment.

On the basis of survey of literature, to the best knowledge of the authors, no report is available on the conservation of *S. chinensis*. During storage in both the male and female cultures, the development of new shoot was accompanied by the senescence of existing shoots on MS (control) as well as on MS supplemented with BA. During the period of conservation, *ca.* 15% cultures contaminated. A significant difference was recorded for the conservation period, between the shoot cultures of male and female. In comparison to control, the conservation

Table 1. Clonal propagation and conservation period of male and female *S. chinensis*

Medium	Male			Female		
MS+BA (µM)	No. of shoots/ culture	Shoot length* (cm)	Conservation period (days)	No. of shoots/ culture*	Shoot length* (cm)	Conservation period (days)
0 (control)	1.7	1.2	150	2.4	1.4	210
2	4.6	1.5	240	3.4	1.7	210
5	5.5	2.0	240	5.0	1.8	240
10	3.0	1.5	270	8.7	1.7	360

* Data were recorded after 90 d from culture

Table 2. Effect of pulse treatment of IBA (50 µM) on the root induction of *S. chinensis* (Culture age: 30 d from transfer on rooting medium)

Time of pulse treatment (min)	Male			Female		
	Rooting culture (%)	No. of roots/ shoot	Root length (cm)	Rooting culture (%)	No. of roots/ shoot	Root length (cm)
0 (control)	0	0	0	0	0	0
2	25	1.5	0.7	25	1.6	1.9
10	27	2.7	0.8	42	3.0	2.6
20	53	3.0	1.5	67	5.0	2.7

period of male shoot cultures increased significantly with the increased concentration of BA and recorded maximum as 270 days on MS + 10 µM BA. The female shoot cultures were found better storer than the male counterparts; as the cultures could survive up to 360 days on the same medium (Table 1).

On the basis of the responsive cultures, 3 shoots/ nodal explants regenerated in 30 days and each shoot bore 3 nodes. On extrapolation of the available data, an average of 1,06,819 female and 84,498 male plantlets can be obtained on MS medium supplemented with 10 µM and 5 µM BA, respectively. Female plants not only produced more number of plantlets than the male ones but also could be conserved for longer duration. The present study describes the simple protocols for clonal propagation and medium-term conservation of jojoba. For long-term conservation, the experiments are in progress to standardize the cryopreservation protocols using encapsulation and dehydration methods.

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Effects of Polyamines on *In Vitro* Conservation of *Vanilla planifolia* (Salisb.) Ames

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Vanilla planifolia, a tropical orchid, belongs to the family Orchidaceae. It is grown for its fruits that yield vanilla flavour used in food, beverages and ice creams *etc.* Although, seed setting occurs in *Vanilla* but the seed viability is very low, therefore, it is propagated vegetatively through cuttings. Plantlet regeneration *via* callus (Davidonis and Knorr, 1991; Philip and Nainar,1986) and without callus in *V. planifolia* has been reported (George and Ravishankar, 1997). Polyamines are a group of ubiquitous, polycationic substances found in the prokaryotic and eukaryotic cells, that influence the cellular and developmental processes (Dey *et al.*, 1998). Embryogenesis, senescence and flowering are reported to be influenced by polyamines in cucumber (Tassoni *et al.*, 1996) and higher catabolism of polyamines in the organogenesis from leaf disc was reported in *Chrysanthemum* (Aribaud *et al.*, 1999). With the assumption that exogenous supply of polyamines will influence the senescence in shoot cultures of *V. planifolia*,

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