JOURNAL OF FOREST SCIENCE, *62*, 2016 (3): 107–115

doi: 10.17221/80/2015-JFS

Enhanced micropropagation protocol of *ex vitro* rooting of a commercially important crop plant *Simmondsia chinensis* (Link) Schneider

A. Singh, P.K. Agarwal

Central Salt and Marine Chemicals Research Institute, Council of Scientific and Industrial Research, Bhavnagar, Gujarat, India

ABSTRACT: A micropropagation protocol was developed by further improvement of prevailing methods using proven germplasm for *ex vitro* rooting and addressed the effect of the number of subcultures on the rooting ability of shoots. A comparative study was done between *in vitro* rooting method and *ex vitro* rooting method. Using the *ex vitro* rooting method a plantlet could be produced in 135 days, which was in a shorter time compared to the *in vitro* rooting method – 180 days. The best axillary shoot bud induction was observed on Murashige and Skoog (MS) medium supplemented with 4.6 µM thidiazuron (TDZ) with 5 shoot buds per explant. In the shoot cluster, which was subcultured on MS medium supplemented with 2.3 µM TDZ, the rate of shoot multiplication increased in the 3rd subculture. The maximum mean number of shoots per explant (20) was obtained at the 3rd subculture on the same medium. Shoots were harvested at the 1st, 3rd and 5th subculture and pulse treated for root induction. The highest rooting (95%) was achieved from the 3rd subculture onwards with pulse treated shoots for fifteen days. The rooted plants could be established in a greenhouse with 99% survival. *Ex vitro* rooting is a promising method to reduce the time for plant generation. The resultant plantlets well established in pots and fruiting was observed within a year.

Keywords: fruiting; genotype; Jojoba

Jojoba (*Simmondsia chinensis*) is a shrub native to the arid regions of Northern Mexico and South Western United States. Jojoba is in very high demand, making it a profitable crop due to its potential application in cosmetic, petroleum, and pharmaceutical industries (Agrawal et al. 2002; Tbares et al. 2004). Jojoba oil is liquid gold from the desert and one of nature's gifts to the human race (Bhardwaj et al. 2010), and it is a desert whale as Jojoba oil is recognized as an alternative to sperm whale oil and has almost the same properties as the oil obtained from the whale sperm (Low, Hackett 1981), which is now listed as an endangered species.

Jojoba can be propagated through seeds but due to low and inconsistent seed yield the cultivations were not taken up as expected (Chaturvedi, Sharma 1989; Coates et al. 2006). Vegetative propagation could be used for the generation of selected clones with limited success (Loreto et al. 1998). In order to overcome the difficulties in vegetative propagation methods for micropropagation have been developed using an *in vitro* rooting method (Chaturvedi, Sharma 1989; Mills et al. 1997; Llorente, Apóstolo 1998; Roussos et al. 1999; Hamama et al. 2001; Tyagi, Prakash 2004; Llorente et al. 2007; Singh et al. 2008). *Ex vitro* rooting is a promising method, skipping the phase of *in vitro* rooting, shortens the micropropagation cycle by 2–3 weeks or more and so minimizes the time period of the plantlet generation (Debergh, Maene 1981; Martin 2003). However, very few attempts have been made in different plants (Bha-

Supported by the Central Salt and Marine Chemicals Research Institute (ProCSIR-CSMCRI), Communication No. 196, and by the Council of Scientific and Industrial Research in New Delhi, Project No. MLP0014.

tia et al. 2002; Martin 2003; Xu et al. 2008; Singh et al. 2014) using this technique and no such attempt has been made in Jojoba.

Many researchers have reported the effect of the number of subcultures on the rooting capacity of microshoots. In many tree species such observations were made e.g. in apple (*Malus pumila* Miller cv. Fuji, Starkrimson), shoots acquired a high rooting ability with increasing the number of subcultures (Chang et al. 1991; Noiton et al. 1992); walnut (*Juglans regia* Linneaus) shoots have a high rooting rate after they have been subcultured for 4 years (Pei et al. 2002; Wang, Guo 2007) when a high rooting rate was found until the 8th subculture. During adventitious root formation endogenous hormones are involved. Indoleacetic acid oxidase (IAAO), peroxidase oxidase (POD) and polyphenol oxidase (PPO) are found to play a role in adventitious root formation (Huang et al. 2002; Xiao et al. 2002; Kochhar et al. 2005). The successful rooting depends upon optimal levels of endogenous phytohormones required for the rooting of that particular plant (Pan, Tian 1999; Wang et al. 2005).

As stated above, the number of subcultures affects the formation of adventitious roots in many species of woody plants. However, no such studies have been done earlier to reveal the effect of successive subcultures of Jojoba microshoots on rooting. Recently, we have developed the large-scale micropropagation protocol from nodal (Singh et al. 2008) and leaf (Singh et al. 2011) explants.

In this study, we report the clonal multiplication of Jojoba using *ex vitro* rooting and effect of the number of subcultures on multiple shoot buds and their rooting ability upon subsequent subcultures.

MATERIAL AND METHODS

Plant material and culture conditions. High-yielding four female (CSMCRI 1-1, CSMCRI 12-8, CSMCRI 10-4 and CSMCRI 20-3) and two male genotypes (Male-1 and Male-2) with an abundance of flowering were selected from the Central Salt and Marine Chemicals Research (CSMCRI) experimental station Zanjmer (21.50°N, 17.53'E, 72.10°N, 44.96'E), Bhavnagar, Gujarat. Actively growing young shoots (5–10 inter nodes) were collected early in the morning and processed for surface sterilization as described by Singh et al. (2008). Uniform culture conditions were applied in all experiments. Murashige and Skoog (1962) medium (MS medium) was supplemented with plant growth regulators (PGRs) in different concentrations and combinations. The pH of the medium was adjusted to 5.8 using 1 N KOH or HCl, prior to autoclaving at a pressure of 105 kPa at 121°C for 20 min. Explants were inoculated into 32 × 200 mm culture tubes containing 40 ml of culture medium which was equally distributed using an automatic dispenser (ASE chenit PM05*)*. The cultures were maintained at 25 ± 2°C under 16 h photoperiod with 35–40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiance (cool white fluorescent tubes).

Axillary bud induction and multiplication. The nodal segments of approximately 2–3 cm length of all genotypes were cultured on MS medium supplemented with TDZ (thidiazuron, 0.46–6.90 μM) and 0.62% agar (w/v, Qualigens, Glaxo Fine Chemicals, Mumbai, India). After 4 weeks, explants were subcultured on MS medium supplemented with different concentrations of TDZ (0.46–6.90 μM) for shoot multiplication and elongation. The basal mass remaining after harvesting the shoots was subcultured onto a fresh shoot multiplication medium for further multiplication. The multiplication rate, shoot elongation and number of shoots per explant were recorded after 5 weeks of culture.

***In vitro* rooting**. Shoots were harvested at different subcultures, 1st, 3rd and 5th, and were pulse treated in LM (½ strength liquid MS medium) supplemented with 49.0 μM indole-3-butyric acid (IBA) and 5.40 μM 1-naphthaleneacetic acid (NAA) for 5 days and subsequently transferred to ½ strength hormone-free MS medium supplemented with charcoal 0.02% (CM) for rooting.

***Ex vitro* rooting and acclimatization**. Grownup shoots obtained from the 3rd subculture onwards from the medium supplemented with 2.3 μM TDZ were used in all the rooting experiments. To determine the rooting efficiency, shoots were pulse treated by placing in a test tube containing a filter paper boat filled with 20 ml of ½ strength MS liquid medium (LM) supplemented with 12.1–49.0 μM IBA, 2.70–8.10 μM NAA, 2.25–13.5 μM 2,4-dichlorophenoxyacetic acid (2,4-D) for 10 and 15 days and transferred to polythene bags (five shoots per bag) containing sterilized sand irrigated with sterile distilled water containing 500 $mg \cdot l^{-1}$ Bavistin (Singh et al. 2010) and covered with transparent plastic bags to maintain humidity. The rooting percentage was recorded after 3 weeks. The rooted plants were transferred to a greenhouse for further hardening and the survival of plants was recorded after 3–4 weeks.

The treatment effects were assessed by SPSS statistical software (IBM, New York, USA). The 0.05 level of probability was used for statistical significance in the analyses.

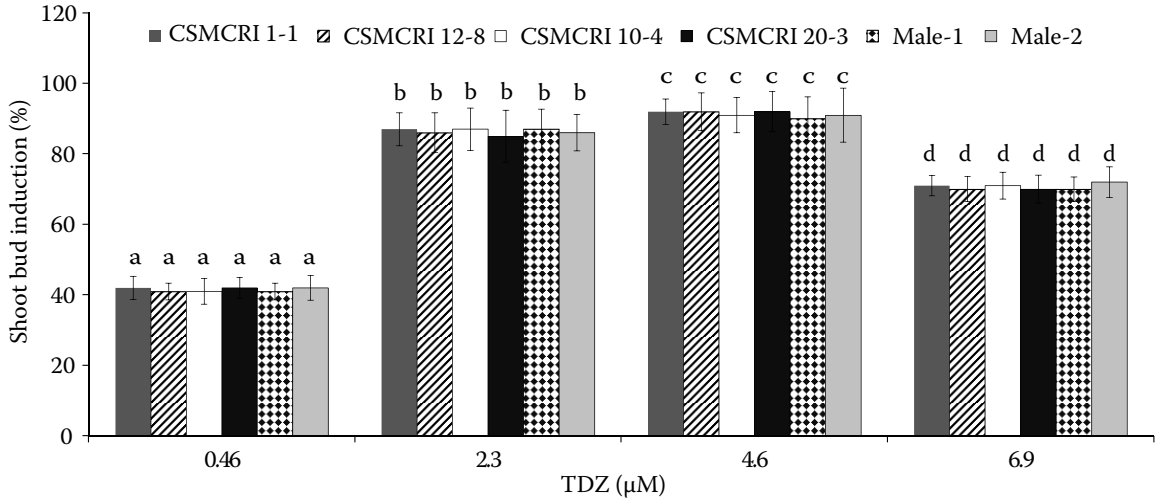

Fig. 1. Effect of thidiazuron (TDZ) on shoot bud induction of different genotypes of *Simmondsia chinensis* (Link) Schneider. The values represent means ± SE of the treatment of 20 explants in three replicate experiments, different letters for TDZ treatments are significantly different at 0.05 probability level (CSMCRI 1-1, CSMCRI 12-8, CSMCRI 10-4, CSMCRI 20-3 – female genotype, Male-1, Male-2 – male genotype)

RESULTS

Effect of TDZ on axillary bud induction, shoot multiplication and elongation

The nodal segments of male and female genotypes cultured on MS medium without PGRs did not show any axillary bud growth, however, the application of 0.46 to 6.90 μM TDZ into the medium induced shoot buds. The best axillary bud induction was recorded at 4.60 μM TDZ (Fig. 1). The induction of shoot buds was more than 90% in all the genotypes. Four weeks after inoculation, positively responding explants (the medium supplemented with 4.60 μM TDZ) were subcultured on MS medium supplemented with 0.46–6.90 μM TDZ for shoot proliferation and elongation. All the concentrations of TDZ facilitated multiple shoot bud induction with a very small amount of callus at the proximal end irrespective of the genotypes studied. The best shoot proliferation and elongation were observed on MS medium supplemented with 2.3 μM TDZ. After the first culture explants of all the genotypes cultured on 2.3 μM TDZ developed 8–10 multiple shoot buds in the first subculture which reached the peak of 14–20 shoots per culture on the 3rd subculture (Figs 2 and 3). The rate of shoot multiplication increased on the third subculture. These shoots attained the growth of 10 to 12 cm and were used for rooting. Axillary buds at the nodes of the in vitro developed shoots also showed 6–8 multiple shoots (Fig. 3c).

In vitro rooting and acclimatization

Shoots from all the genotypes were harvested from the 1st, 3rd and 5th subculture and were pulse treated

Table 1. Effect of subcultures on *in vitro* rooting percentage of different genotypes of *Simmondsia chinensis* (Link) Schneider over 4 weeks

Genotype	Number of subculture		
	1st	3rd	5th
Female			
CSMCRI 1-1	40.12 ± 1.12	94.21 ± 2.29	92.28 ± 2.26
CSMCRI 12-8	39.15 ± 1.16	92.28 ± 2.56	93.24 ± 2.56
CSMCRI 10-4	39.26 ± 1.11	93.16 ± 2.46	95.18 ± 2.29
CSMCRI 20-3	40.24 ± 1.29	92.14 ± 2.48	92.11 ± 1.35
Male			
Male-1	40.16 ± 1.56	95.21 ± 2.35	94.21 ± 2.45
Male-2	41.21 ± 1.34	94.19 ± 2.31	95.16 ± 2.41

values represent means ± SE of the treatment of 20 explants in three replicate experiments

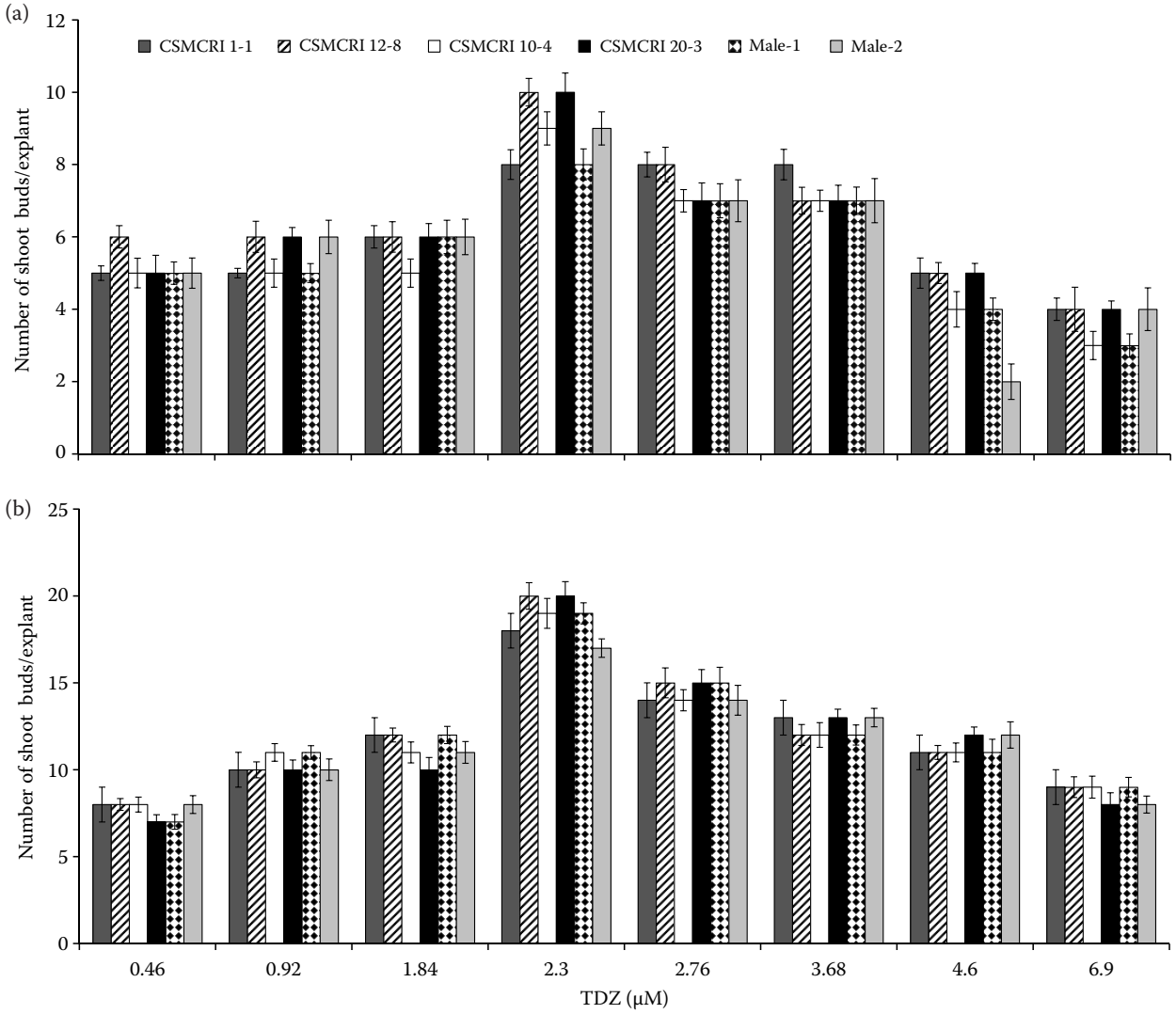

Fig. 2. Effect of the number of subcultures on the number of shoot buds, first subculture (a), third subculture (b) on different genotypes of *Simmondsia chinensis* (Link) Schneider (CSMCRI 1-1, CSMCRI 12-8, CSMCRI 10-4, CSMCRI 20-3 – female genotype, Male-1, Male-2 – male genotype, TDZ – thidiazuron)

in LM for 5 days and then transferred to CM for rooting. The rooting percentage of shoots in all the genotypes was very close to each other and did not vary very much. The highest percent rooting was achieved in the range of 92 to 95% from the 3rd and 5th subculture shoots in all the genotypes (Fig. 3, Table 1). From the third subculture onwards, the rooting percentage was high as compared to the 1st subculture (39–41%). The results reveal that the rooting ability of shoots increased from the 3rd subculture onwards. The shoots in the control failed to root in CM. *In vitro* hardened plants were transferred to a greenhouse for further hardening and 91% survival was achieved (Fig. 4).

Ex vitro rooting and acclimatization

During *in vitro* rooting experiments, we obtained better rooting using shoots from the 3rd culture onwards in all the genotypes. Hence, for *ex vitro* rooting shoots were arbitrarily selected from all the genotypes from the 3rd subculture onwards. In shoots subjected to pulse treatment in LM for 15 days with IBA (24.5 μM) alone 68% rooting with 1.5 ± 0.1 cm root length was achieved. The addition of 2,4-D (4.5 μM) into the medium enhanced rooting up to 95% with the root length of 6.1 ± 0.2 cm (Table 2, Fig. 3). 2,4-D and IBA have synergistic effects not only on rooting but also on root length. The root length of pulse treated shoots for 10 days was 4.2 cm and in 15 days it was 6.1 cm; hence 15-day pulse treatment was optimum for ex vitro rooting and establishment of plants. Shoots in the control failed to root. The high rooting ability of the 3rd subculture shoots was conferred by *ex vitro* rooting results as rooting percentages obtained by both the methods were quite similar (> 90%). *Ex vitro* rooted plants were further hardened in a green-

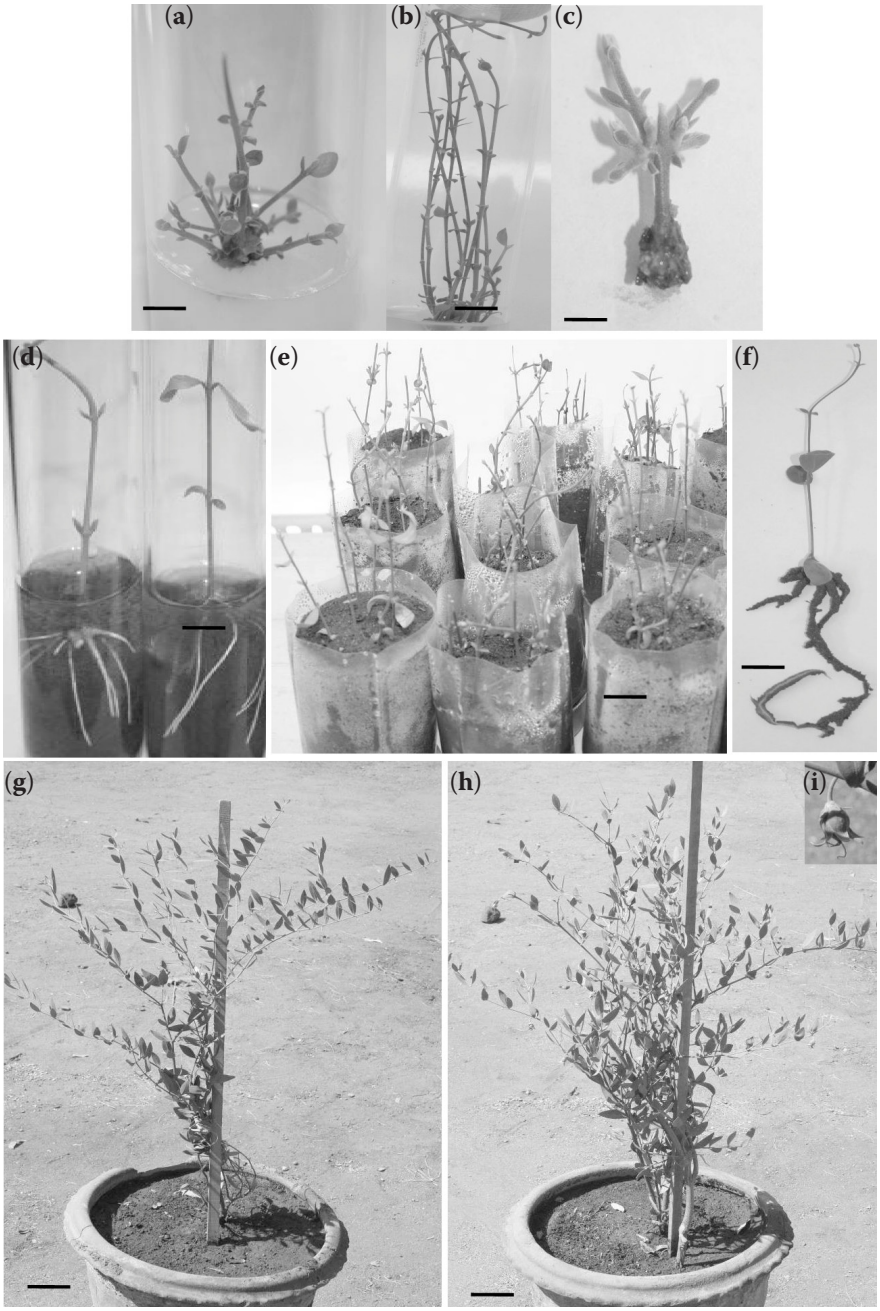

Fig. 3. Micropropagation of Simmondsia chinensis (Link) Schneider using nodal segments: multiple shoot bud induction upon 1st subculture (bar = 1.0 cm) (a), generation and elongation of 17–20 shoot buds on Murashige and Skoog (MS) medium supplemented with 2.3 μM thidiazuron (TDZ) (bar = 1.0 cm) (b), multiple shoot bud induction from axillary buds of *in vitro* shoots on MS medium supplemented with 2.3 μM TDZ (bar = 1.5 cm) (c), *in vitro* rooted shoots in ½ strength hormone-free MS medium supplemented with charcoal 0.02% (bar = 1.5 cm) (d), pulse treated shoots (4–5) placed in a single poly bag containing sterile sand for *ex vitro* rooting covered with poly bag, *ex vitro* rooted plants in bags after 3 weeks (bar = 2.0 cm) (e), *ex vitro* rooted plant removed from the bag after 4 weeks (f), flowering resulted into successful fruiting (bar = 5.0 cm) (g, h), and *ex vitro* rooted plants flowered within a year (i)

house with 99% survival rate. No visual morphological abnormalities were observed in generated plants. *Ex vitro* rooted plants flowered within a year (Fig. 3) and flowering resulted in successful fruiting (Fig. 3).

In the comparative study of these rooting methods, the number of roots developed through *in vitro* rooting was higher (1–15) as compared to *ex vitro* (4–5) rooted shoots. Roots developed *ex vitro* were long and with the secondary root system without any callus at the base. The total time taken for plantlet generation through *ex vitro* rooting was 135 days and was very much shorter as compared to *in vitro* rooting 180 days (Fig. 4).

Table 2. Effect of hormones on *ex vitro* rooting of *Simmondsia chinensis* (Link) Schneider

Hormones (μM)			Pulse treatment (days)			
			10		15	
IBA	NAA	2,4-D	rooting (%)	root length (cm)	rooting (%)	root length (cm)
12.2	–	–	33 ± 2.1^{c}	0.5 ± 0.1^{a}	39 ± 1.4^{a}	0.6 ± 0.1^{a}
24.5	–	–	72 ± 1.2^{f}	1.0 ± 0.1^{b}	68 ± 1.2^{d}	1.5 ± 0.1^{b}
49.0	–	–	61 ± 1.2^{d}	0.6 ± 0.1^{a}	65 ± 1.2^{d}	0.8 ± 0.1^{a}
–	2.70	–	10 ± 1.3^{a}	0.6 ± 0.1^{a}	39 ± 1.2^{a}	0.6 ± 0.1^{a}
–	5.40	–	32 ± 1.8^{c}	0.8 ± 0.1^{a}	40 ± 1.2^{a}	0.9 ± 0.1^{b}
–	8.10	–	21 ± 1.1^{b}	1.7 ± 0.1^{a}	38 ± 1.1^{a}	0.7 ± 0.2^{a}
–	–	2.25	31 ± 1.9^{c}	1.2 ± 0.2^{b}	43 ± 1.9^{b}	1.2 ± 0.2^{b}
–	–	4.50	42 ± 1.8^{d}	1.5 ± 0.2^{b}	49 ± 2.8^{c}	1.6 ± 0.1^{b}
–	–	13.5	35 ± 1.7^{c}	1.4 ± 0.1^{b}	45 ± 1.6^{b}	1.5 ± 0.1^{b}
24.5	–	2.25	71 ± 1.5^{f}	3.8 ± 0.1^{b}	90 ± 2.3^{d}	5.2 ± 0.1^{b}
24.5	–	4.50	73 ± 1.6^{f}	4.2 ± 0.1^{c}	95 ± 2.2^{f}	6.1 ± 0.2^{c}
24.5	–	13.5	70 ± 1.4^{f}	4.1 ± 0.2^{c}	91 ± 2.1^{d}	6.0 ± 0.1^{c}

values represent means ± SE of the treatment of 20 explants in three replicate experiments, means followed by different letters are significantly different at 0.05 probability level; IBA – indole-3-butyric acid, NAA – 1-naphthaleneacetic acid, 2,4-D – 2,4-dichlorophenoxyacetic acid

DISCUSSION

A non-purine phenylurea derivative TDZ is widely used for shoot development (Malik, Saxena 1992; Bhagwat, Lane 2004). It is found to mimic cytokinin-like activity, and to promote release of lateral buds from dormancy (Wang et al. 1986). It has also been reported as a potential plant growth regulator to induce a high frequency of shoot regeneration, particularly in woody plant species (Huetteman, Preece 1993) but no attempts have been made in Jojoba, a typical hardwood plant. In the present investigation, axillary bud induction was observed in the medium supplemented with 4.60 μM TDZ, however, induced shoot buds failed to elongate at the same TDZ concentration. Similar results were reported in chili pepper by Hyde and Phillips (1996). Upon subsequent subcultures, 20 shoots per explant with 14–15 cm length were obtained on MS medium containing 2.30 μM TDZ after 5 weeks of culture. Similar results were reported in *Camellia sinensis* Linnaeus and *Musa acuminata* Colla (Mondal et al. 1998; Farahani et al. 2008). The effect of alone 6-benzylaminopurine (BAP) and in combination with auxin on micropropagation of Jojoba has been documented by different rates of shoot multiplication (Driver, Kuniyuki 1984; Chaturvedi, Sharma 1989; Mills et al. 1997; Llorente, Apóstolo 1998; Tyagi, Prakash 2004; Singh et al. 2008). In the present study, TDZ alone could give rise to 20 shoots. Such a response may be due to an increase in the level of endogenous cytokinins. Apart from its cytokinin-like activity, it has been suggested to be a modulator of the endogenous auxin levels (Murthy et al. 1995). This may be the reason why during the present study TDZ might

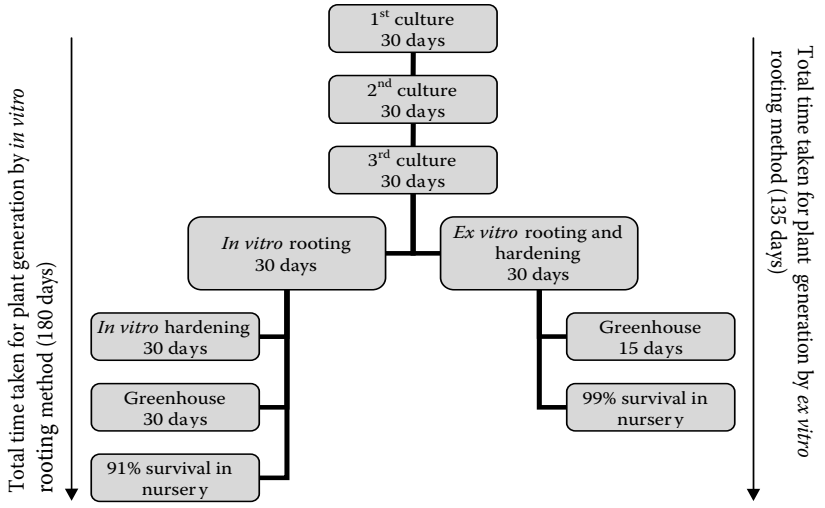

Fig. 4. Schematic representation of time taken for plantlet generation using *in vitro* and *ex vitro* rooting methods of *Simmondsia chinensis* (Link) Schneider

have scored higher numbers of shoots. BAP is reported to produce a higher number of shoots per explant, however, it induces callus and hyperhydrated shoots (Llorente, Apóstolo 1998, 2000; Mills et al. 2004; Tyagi, Prakash 2004).

We could achieve more than 90% in vitro rooting, which was higher than earlier (Chaturvedi, Sharma 1989; Mills et al. 1997; Tyagi, Prakash 2004). The rooting percentage of shoots increased from around 39 to 92% from the first to the third subculture, hence from the 3rd culture onwards shoots are more suitable for rooting, which may be due to the better physiological condition of shoots for better rooting. Similar results were observed in *Eucalyptus* L'Heritier (Goncalves 1980; Gupta et al. 1981; Badia 1982; Mccomb, Bennett 1982). Also in perennial plants/tree species the growth of the culture may be slow as compared to annual plants, and restore their juvenile traits essential for rooting upon subsequent subcultures, hence this may be one of the reasons for a high rooting percentage in older cultures/3rd subculture onwards. A progressive increase in the rooting ability of chestnut microshoots occurred at the 8th subculture (Feijó, Pais 1990; Wang, Guo 2007). In Chinese chestnut (*Castanea mollissima* Blume) it was observed that upon subculture the levels of endogenous indoleacetic acid (IAA) in microshoots gradually increased, and endogenous levels of abscisic acid, cytokinin and gibbrelic acid in microshoots decreased. The level of 3-IAA was directly correlated with subculture numbers and rooting rates in the chestnut plant (Hou et al. 2010). In all the genotypes rooting ability was very close to each other, which may be due to similar growth regulator uptake, translocation rate and metabolic processes among genotypes (Blakesley 1991). Similarly to in vitro rooting, high *ex vitro* rooting (> 90%) was achieved from the shoots of the 3rd subculture onwards. However, shoots generated according to Singh et al. (2008) resulted in 82.5% *ex vitro* rooting (data not shown). *Ex vitro* rooting using IBA in combination with 2,4-D was found to be best as compared to alone IBA; similar results were observed in Stachousia tryonii (Bhatia et al. 2002). Rooting in sand or in auxin-free medium corroborates other reports that although auxins are essential for root induction, they may not be required for root growth and their continued presence may even inhibit the root growth (Singh et al. 2008).

Ex vitro rooted plantlets are usually more suited for transplantation as compared to *in vitro* rooted plantlets (Andersen 1986). The root system developed *ex vitro* was different from the root system developed during *in vitro* rooting practice. The *ex vitro* rooting method was also found to be better for the survival and establishment of the plant as compared to the *in vitro* rooting (Singh et al. 2011, 2014).

We report here rooting in one step through *ex vitro* rooting to minimize time and labour for plantlet generation. Numerous studies have addressed *in vitro* rooting in this plant to the best of our knowledge till now but there is no report on *ex vitro* rooting of micropropagated shoots of *S. chinensis* male and female plants. The study clearly figured out the effect of subculture on rooting efficiency and also found that the rooting pattern of *ex vitro* rooting is preferable for the better establishment and survival of the plant. This protocol will be useful for mass propagation in a short time.

References

Agrawal V., Prakash S., Gupta S.C. (2002): Effective protocol for *in vitro* shoot production through nodal explants of *Simmondsia chinensis*. Biologia Plantarum, 45: 449–453.

Andersen A.S. (1986): Environmental influences on adventitious rooting in cuttings of non-woody species. In: Jackson M.B. (ed.): New Root Formation in Plants and Cuttings. Dordrecht, Martinus Nijhoff: 223–253.

Apóstolo N.M., Llorente B.E. (2000): Anatomy of normal and hyperhidric leaves and shoots of *in vitro* grown *Simmondsia chinensis* (Link) Schneider. In Vitro Cellular and Developmental Biology – Plant, 36: 243–249.

Badia N.K. (1982): *Eucalyptus rudis* Endl.: Techniques de micropropagation par la culture de noeuds *in vitro*. In: Colloque International sur la Culture *in vitro* des Essences Forestières, IUFRO, AFOCEL, Nangis, France: 135–142.

Bhagwat B., Lane W.D. (2004): *In vitro* shoot regeneration from leaves of sweet cherry (*Prunus avium*) 'Lapins' and 'Sweetheart'. Plant Cell and Tissue Culture, 78: 173–181.

Bhardwaj M., Uppal S., Jain S., Kharb P., Dhillon R., Jain R.K. (2010): Comparative assessment of ISSR and RAPD marker assays for genetic diversity analysis in Jojoba [*Simmondsia chinensis* (Link) Schneider]. Journal of Plant Biochemistry and Biotechnology, 19: 255–258.

Bhatia N.P., Bhatia P., Ashwath N. (2002): *Ex vitro* rooting of micropropagated shoots of *Stackhousia tryonii*. Biologia Plantarum, 45: 441–444.

Blakesley A. (1991): Uptake and metabolism of 6-benzyladenine in shoot proliferation of *Musa* and *Rhododendron*. Plant Cell and Tissue Culture, 25: 69–74.

Chang Y., Chen S., Ma B., Lu Z. (1991): Studies of the relationship between some hormones and adventitious root formation in shoot-tip culture of apple (*Malus pumila* mill cvs). Journal of Agricultural University of Hebei, 14: 1–5.

Chaturvedi H.C., Sharma M. (1989): *In vitro* production of cloned plants of Jojoba [*Simmondsia chinensis* (Link) Schneider] through shoot proliferation in long term culture. Plant Science, 63: 199–207.

Coates W., Ayerza R., Palzkill D. (2006): Supplemental pollination of Jojoba – a means to increase yields. Industrial Crops and Products, 24: 41–45.

Debergh P.C., Maene L.J. (1981): A scheme for the commercial propagation of ornamental plants by tissue culture. Scientia Horticulture, 14: 335–345.

Driver J.A., Kuniyuki A.H. (1984): *In vitro* propagation of paradox walnut rootstock. Horticulture Science, 19: 507–509.

Farahani F., Aminpoor H., Sheidai M., Noormohammadi Z., Mazinani M.H. (2008): An improved system for *in vitro* propagation of Banana (*Musa acuminate* L.) Cultivars. Asian Journal of Plant Science, 7: 116–118.

Goncalves A.N. (1980): Reversion to juvenility and cloning of *Eucalyptus urophyllu* S.T. Blake in cell and tissue culture systems. In: IUFRO Symposium and Workshop on Genetic Improvement and Productivity of Fast Growing Tree Species, Sao Paulo, Brazil, Aug 25–30, 1980: 786–787.

Gupta P.K., Mascarenhas A.F., Jagannathan V. (1981): Tissue culture of forest trees – clonal propagation of *Eucalyptus citriodora* Hook. by tissue culture. Plant Science Letter, 20: 195–201.

Hamama L., Baaziz M., Letouze R. (2001): Somatic embryogenesis and plant regeneration from leaf tissue of Jojoba. Plant Cell Tissue and Organ Culture, 65: 109–113.

Hofer M. (1995): *In vitro* androgenesis in apple. Gartenbauwissenschaft, 60: 12–15.

Huang Z., Li M., Tan S. (2002): Study on the relationship between the changes of activities and isoenzymes of polyphenol oxidase and the rooting of *Eucalyptus* cuttings after treatment with indolebutyric acid. Guihaia, 21: 77–82.

Huetteman C.A., Preece J.E. (1993): Thidiazuron a potent cytokinin for woody plant tissue culture. Plant Cell and Tissue Organ Culture, 33: 105–119.

Hyde C.L., Phillips G. (1996): Silver nitrate promotes shoot development and plant regeneration of Chile pepper (*Capsicum annuum* L.) via organogenesis. In Vitro Cellular and Developmental Biology – Plant, 32: 72–80.

Hou J.W., Guo S.J., Wang G.Y. (2010): Effects of *in vitro* subculture on the physiological characteristics of adventitious root formation in microshoots of *Castanea mollissima* cv. 'yanshanhong'. Journal of Forestry Research, 21: 155–160.

Kochhar S., Kochhar V.K., Singh S.P., Katiyar R.S., Pushpangadan P. (2005): Differential rooting and sprouting behaviour of two *Jatropha* species and associated physiological and biochemical changes. Current Science, 89: 936–939.

Llorente B.E., Apóstolo N.M. (1998): Effect of different growth regulators and genotype on *in vitro* propagation of Jojoba. New Zealand Journal of Crop and Horticulture Science, 26: 55–62.

Llorente B.E., Juarez M.L., Apóstolo M.N. (2007): Exogenous trehalose affects morphogenesis *in vitro* of Jojoba. Plant Cell Tissue and Organ Culture, 89: 193–201.

Loreto P., Botti C., Palzkill D. (1998): Rooting of Jojoba cuttings: The effect of clone, substrate composition and temperature. Industrial Crops and Products, 9: 47–52.

Low C.B., Hackett W.P. (1981): Vegetative propagation of Jojoba. California Agriculture, 35: 12–13.

Malik K.A., Saxena P.K. (1992): Regeneration in *Phaseolus vulgaris* L. High-frequency induction of direct shoot formation in intact seedlings by N6-benzylaminopurine and thidiazuron. Planta, 186: 384–389.

Martin K.P. (2003): Rapid *in vitro* multiplication and *ex vitro* rooting of *Rotula aquatica* Lour., a rare rhoeophytic woody medicinal plant. Plant Cell Report, 21: 415–420.

McComb J.A., Bennett I.J. (1982): Vegetative propagation of eucalypts: Using tissue culture and its application to forest improvement in Western Australia. In: Fujiwara A. (ed.): Proceedings of the 5th International Congress of Plant Tissue and Cell Culture, Tokyo, Japan, July 11–16, 1982: 721–722.

Mills D., Wenkart S., Benzioni A. (1997): Micropropagation of *Simmondsia chinensis* (Jojoba). In: Bajaj Y.P.S. (ed.): Biotechnology in Agriculture and Forestry. Berlin-Heidelberg, Springer-Verlag: 380–939.

Mills D., Yanqing Z., Benzioni A. (2004): Improvement of Jojoba shoot multiplication *in vitro* by ventilation. In Vitro Cell and Developmental Biology – Plant, 40: 396–402.

Mondal T.K., Bhattacharya A., Sood A., Ahuja P.S. (1998): Micropropagation of tea (*Camellia sinensis* (L.) O. Kuntze) using Thidiazuron. Plant Growth Regulation, 5: 57–61.

Murashige T., Skoog F. (1962): A revised medium for rapid growth and bio assays with tobacco issue cultures. Physiologia Plantarum, 15: 473–497.

Murthy B.N.S., Murch S.J., Saxena P.K. (1995): Thidiazuron induced somatic embryogenesis in intact seedlings of peanut (*Arachis hypogaea* L.), endogenous growth regulator levels and significance of cotyledons. Physiologia Plantarum, 94: 268–276.

Noiton D., Vine J.H., Mullins M.G. (1992): Effects of serial subculture *in vitro* on the endogenous levels of indole-3-acetic acid and root ability in microcuttings of 'Jonathan' apple. Plant Growth Regulation, 11: 377–383.

Pan R., Tian X. (1999): Comparative effect of IBA, BSAA and 5,6-Cl_2-IAA-Me on the rooting of hypocotyl in mung bean. Plant Growth Regulation, 27: 91–98.

Pei D., Yuan L., Xi S., Gu R. (2002): Shoot rooting *in vitro* for walnut cultivars. Scientia Silvae Sincae, 38: 32–38.

Roussos P.A., Tolia-Marioli A., Pontikis C.A., Kotsias D. (1999): Rapid multiplication of Jojoba seedlings by *in vitro* culture. Plant Cell Tissue and Organ Culture, 57: 133–137.

Singh A., Reddy M.P., Chikara J., Singh S. (2010): A simple regeneration protocol from stem explants of *Jatropha curcas* – A biodiesel plant. Industrial Crops and Products, 31: 209–213.

Singh A., Reddy M.P., Patolia J.S. (2008) An improved protocol for micropropagation of elite genotypes of *Sim-*

mondsia chinensis (Link) Schneider. Biologia Plantatum, 52: 538–542.

Singh A., Reddy M.P., Chikara J. (2011): Shoot regeneration from petiole explants of *Simmondsia chinensis* (Link) Schneider. The Jounal of Horticulture Science and Biotechnology, 86: 153–158.

Singh A., Jani K., Sagervanshi A., Agrawal P.K. (2014): High-frequency regeneration by abscisic acid (ABA) from petiole callus of *Jatropha curcas*. In Vitro Cell and Developmental Biology – Plant, 50: 638–645.

Tobares L., Frati M., Guzman C., Maestri D. (2004): Agronomical and chemical traits as descriptors for discrimination and selection of Jojoba (*Simmondsia chinensis*) clones. Industrial Crops and Products, 19: 107–111.

Tyagi R.K., Prakash S.S. (2004): Genotype and sex-specific protocol for *in vitro* micropropagation and medium-term conservation of Jojoba. Biologia Plantarum, 48: 119–123.

Wang G., Guo S. (2007): The key factor of tip culture of *Castanea mollissima* cv. 'yanshanhong'. Journal of South China Agriculture University, 28: 144–147.

Wang J.X., Yan X.L., Pan R.C. (2005): Relationship between adventitious root formation and plant hormones. Plant Physiology Communications, 41: 133–142.

Wang S.Y., Steffens G.L., Faust M. (1986): Breaking bud dormancy in apple with a plant bioregulator, thidiazuron. Phytochemistry, 25: 311–317.

Xiao G., Yang Q. (2002): Advances in endogenous hormones of plant tissue culture. Journal of Yunnan Agriculture University, 62: 271–273.

Xu J., Wang Y., Zhang Y., Chai T. (2008): Rapid in vitro multiplication and ex vitro rooting of Malus zumi (Matsumura) Rehd. Acta Physiologiae Plantarum, 30: 129–132.

Received for publication August 31, 2015
Accepted after corrections January 25, 2016

Corresponding author:

Dr. Aneesha Singh, Ph.D, Central Salt and Marine Chemicals Research Institute, Council of Scientific and Industrial Research, G.B Marg, Bhavnagar-364002, Gujarat, India; e-mail: aneesha.singh@rediffmail.com