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ORIGINAL PAPER

Induction of somatic embryogenesis from female flower buds of elite *Schisandra chinensis*

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Abstract Somatic embryogenesis (SE) was induced in female flower buds from mature *Schisandra chinensis* cultivar 'Hongzhenzhu'. Somatic embryo structures were induced at a low frequency from unopened female flower buds and excised unopened on Murashige and Skoog (MS) agar medium containing 4.0 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D). Friable embryogenic calli were induced from somatic embryo structures after three to four subcultures on initiation medium. The frequencies of mature somatic embryo germination and plantlet conversion were low, but increased in the presence of gibberellic acid (GA_3). Some germinated somatic embryos could form friable embryogenic calli on medium without plant growth regulators (PGRs). The germination and conversion frequencies of somatic embryos from embryogenic calli induced using PGR-free medium were higher than for somatic embryos from embryogenic calli induced on medium containing 2,4-D. Most somatic embryos from 2,4-D-induced embryogenic calli had trumpet-shaped embryos, and most somatic embryos from PGR-free medium–induced embryogenic calli had two or three cotyledons. Histological observation indicated that two- and three-cotyledon embryos had defined shoot primordia, but most of the trumpet-shaped embryos yielded plantlets that lacked or had poorly developed meristem tissue. Cytological and random amplification of polymorphic DNA (RAPD) analyses indicated no evidence of genetic variation in the plantlets of somatic embryo origin.

Keywords Woody vine · Secondary somatic embryo · Elite tree · Ploidy · Genetic variation · Magnoliaceae

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Introduction

Schisandra chinensis Baill (Magnoliaceae) is a woody, deciduous vine mainly found in Northeast China, Korea, Japan, and the Far East of Russia (Hancke et al. 1999). The fruits are used in traditional Chinese medicine and are also widely used in the pharmaceutical, wine, cosmetics, and health food industries. Due to forest reduction and extensive environmental damage, wild resources of the plant have been abruptly reduced and cannot meet market demand. *S. chinensis* breeding has been performed in China since the early 1980s. As a result, the first cultivar, 'Hongzhenzhu', was selected from wild populations and bred in 2001 at the Chinese Academy of Agricultural Sciences Institute. 'Hongzhenzhu' is a high-yield cultivar, with average yield of 0.5, 1.3, and 2.2 kg of fruit from 3-, 4-, and 5-year old trees, respectively. However, the propagation success is very low using conventional vegetative propagation methods, such as cuttings. Currently, *S. chinensis* is mainly propagated by seed. However, cultivated seed populations are rarely homogeneous and do not ensure good fruit quality and high yield. Establishment of an efficient method for micropropagation of the cultivar is urgently needed.

Clonal propagation through somatic embryogenesis (SE) can shorten the time needed for breeding and can improve the

uniformity and quality of nursery stock (Stasolla and Yeung 2003). In many plant species, immature zygotic embryos are commonly used as explants for induction of SE. However, seed populations vary widely, and transmission of desirable traits from the parental tree is not reliable. Therefore, it is necessary to choose vegetative organs of selected elite mature trees as starting materials. However, embryogenic cultures in woody perennials are often difficult to initiate from plants beyond the seedling stage, especially when derived from mature trees (Bonga et al. 2010). Only a few studies have been published describing somatic embryo induction from mature tree explants.

For plant propagation, success in micropropagation depends on the absence of somaclonal propagation in clonally propagated plants (Mallón et al. 2010; Mishra et al. 2011). Unfortunately, some in vitro regenerated plantlets exhibit somaclonal variation, which has been demonstrated to be heritable and therefore undesirable in somatic clones. Therefore, detection of such variation early in the life of a plant is required to avoid economic disaster during commercial regeneration. Chromosomal alterations, which generate variation in somatic embryo-derived plants, can be detected by flew cytometry (Tremblay et al. 1999; Yang et al. 2010a).

Currently, molecular methods provide valuable tools for determination of clonal identity. The most widely used procedure for evaluating patterns of existing genetic variations are molecular methods such as random amplified polymorphic DNA (RAPD) analysis, because it is quick, easy to perform, and does not require radioactive materials (Mallón et al. 2010; Mishra et al. 2011). RAPD analysis has previously been used for certification of genetic stability of embryogenic systems in some tree species (Tang 2001).

Kim et al. (2005) and Smiskova et al. (2005) both reported induction of SE from immature zygotic embryos of *S. chinensis*. Recently, we obtained somatic embryos from hypocotyl and cotyledon explants of germinated *S. chinensis* zygotic embryos (Chen et al. 2010). There are no published reports describing SE from mature *S. chinensis* vines. The current study describes the development of a protocol for SE and plant conversion from female flower bud explants of *S. chinensis*. We analyzed the ploidy levels of somatic embryo–derived plants using flow cytometry and assessed clonal fidelity with RAPD.

Materials and methods

Plant material and induction of SE

Schisandra chinensis cultivar 'Hongzhenzhu' ('Red pearl') was used in this study. In late April 2005, 2-year-old branches having mostly female flower buds and vegetative buds were collected from 5-year-old cutting-propagated elite plants in an orchard at the Institute of Special Wild Economic Animal and Plant Science, Chinese Academy of Agricultural Sciences, Jilin, China. Branch cuttings from these trees were placed in a 1-l Erlenmeyer flask with 400 ml sterile distilled water at 25°C under 45 μmol m^{-2} s^{-1} illumination with cool white fluorescent lights. Water was replenished at 4-day intervals. Female flower buds swelled after 4–5 days in culture (Fig. 1a). Recently swollen, unopened flower buds and open vegetative buds with expanded leaves were collected after 1–2 weeks. Excised buds from which two or three outer scales had been removed were washed in running tap water for 12 h, soaked in 70% (v/v) ethanol for 1 min, sterilized with 0.1% (w/v) $HgCl_2$ solution containing two drops of Tween-20 for 12 min, and then rinsed five times with sterile distilled water. Intact female flower buds and excised bud leaves were cultured on MS (Murashige and Skoog 1962) agar medium containing 3% (w/v) sucrose and 4.0 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) (Sigma, MO, USA).

All media were adjusted to pH 5.8 before adding 8.0 g l^{-1} plant agar (Duchefa, Haarlem, The Netherlands) and were then sterilized by autoclaving at 1.1 kg cm^{-2} (121°C) for 20 min. Cultures were grown in 150-ml Erlenmeyer flasks containing 30 ml medium and were subcultured at 4-week intervals. The culture room was maintained at 25 ± 2°C with a 16-h photoperiod of 36 μmol m^{-2} s^{-1} (cool white fluorescent tubes).

Establishment of recurrent somatic embryogenic cultures

Somatic embryo structures that had formed from explants were transferred to the same medium as used for the primary culture. After 12 weeks in culture, the frequency of embryogenic callus formation was determined. For proliferation, about 15 mg actively growing embryogenic callus was cultured on MS medium containing 3.0% (w/v) sucrose and 0, 1.0, or 4.0 mg l^{-1} 2,4-D.

For somatic embryo development and plantlet conversion, about 15 mg embryogenic callus was cultured on 1/3-strength MS medium containing 2.0% (w/v) sucrose. After 4 weeks, somatic embryos (mainly at the cotyledon stage) were transferred to medium containing 0, 1.0, 5.0 or 10.0 mg l^{-1} gibberellic acid (GA_3) and were cultured for 1, 5, 10 or 15 days. After the GA_3 treatment, somatic embryos from the same flask were transferred to another flask containing 1/3-strength MS medium with 2.0% (w/v) sucrose. Six germinated somatic embryos were cultured per flask. Each experimental unit consisted of five flasks with three replicates.

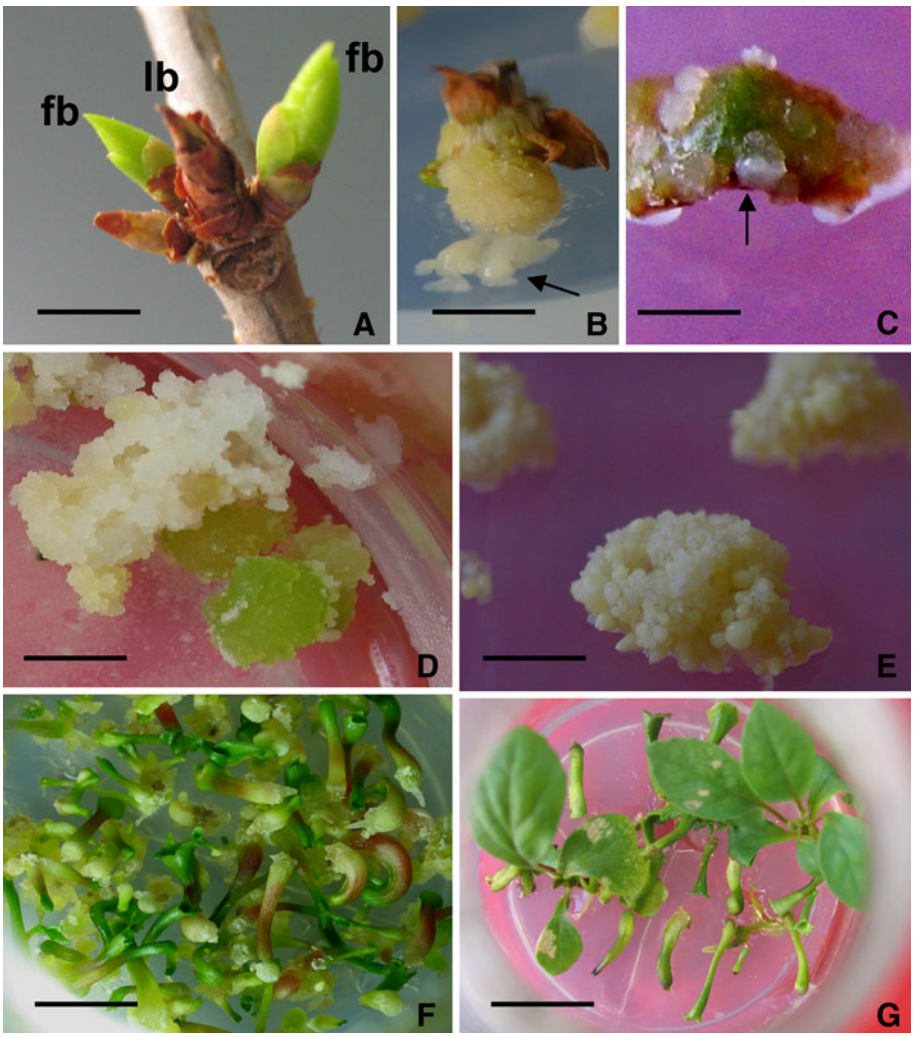

Fig. 1 Somatic embryogenesis from female flower buds of mature *Schisandra chinensis* trees of cultivar 'Hongzhenzhu'. **a** Flower bud collected in early spring. *Bar* 13 mm. **b** Somatic embryos induced from a flower bud–derived callus on MS medium supplemented with 4.0 mg l^{-1} 2,4-D. *Bar* 5 mm. **c** Somatic embryos induced from flower bud derived unopened leaf explants on MS medium supplemented with 4.0 mg l^{-1} 2,4-D. *Bar* 1 mm. **d** Embryogenic calli induced and proliferated from somatic embryos on MS medium supplemented with 4.0 mg l^{-1} 2,4-D. *Bar* 1 mm. **e** Numerous globular somatic embryos induced from an embryogenic callus on 1/3-strength MS medium containing 2% sucrose. *Bar* 2 mm. **f** Mature somatic embryos. *Bar* 8 mm. **g** Plantlets from germinated somatic embryos. *Bar* 10 mm. *fb* flower bud, *lb* leaf bud. *Arrows* indicate somatic embryos

To generate a new cycle of secondary SE, germinated somatic embryos that failed to convert into plants were transferred into 1/3-strength MS medium containing 0, 0.1, 1.0 or 4.0 mg l^{-1} 2,4-D. Ten germinated embryos were cultured per flask, and ten replicates of each treatment were prepared. After 8 weeks, the embryogenic callus induction frequency was determined. For somatic embryo development, embryogenic calli induced on medium without plant growth regulators (PGRs) or with 1.0 mg l^{-1} 2,4-D were transferred to 1/3-strength MS medium containing 2.0% sucrose. For germination, mature somatic embryos were cultured for 5 days in 1/3-strength MS medium containing 1.0 mg l^{-1} GA_3 and were then transferred to the same germination medium without PGRs. After 4 weeks, germinated embryos were selected and transferred to conversion medium. The composition of both germination and conversion medium was the same as that of the primary culture medium.

Ploidy analysis

Nuclear suspensions of somatic embryo-derived plants and parental plants were prepared as described (Yang et al. 2010a). Ploidy determination was performed using the PA-I ploidy analyzer (Partec, Munster, Germany).

Genomic DNA isolation and random amplification of polymorphic DNA (RAPD) analysis

Twenty randomly selected SE-derived plantlets, 20 seed-propagated plantlets, and four parental plants were subjected to RAPD analysis. Total genomic DNA was isolated following the modified Murray and Thompson (1980) method using cetyltrimethylammonium bromide (CTAB) (Sigma, MO, USA). Two primers (OPA-17 and OPJ-6) selected from 55 oligonucleotide primers (OPA 1–19, OPB 1–11, OPC 6–15, OPD 16–20, and OPJ 1–10; Operon

Technologies, Alameda, CA, USA) were used to identify polymorphisms. Polymerase chain reaction (PCR) mixtures (20 µl) contained 50 ng template DNA, 30 ng primer, 0.2 mM dNTP, 1× PCR buffer (Mg^{2+}), and 1 unit Taq polymerase (Takara, Shiga, Japan). Amplification was performed with an initial denaturation step at 94°C for 5 min followed by 40 cycles at 94°C for 1 min, 37°C for 45 s, and 72°C for 1 min, with final extension at 72°C for 7 min. The reaction products were subjected to 0.8% (w/v) agarose gel electrophoresis (Sub-Cell GT; Bio-Rad), stained with ethidium bromide, and imaged under ultraviolet light using a digital imaging system (UltraCam; Syngene, Cambridge, UK).

Plant growth

Fifty somatic plantlets with well-developed leaves and roots were selected and transferred to pots containing autoclaved sand and soil (1:3 mixture). Pots were covered with perforated polythene bags to maintain high humidity. The covers were removed after 3 weeks when new leaves developed. Plants were grown at ~21°C with a 16-h photoperiod of 36 µmol m^{-2} s^{-1} (cool white fluorescent tubes). The survival frequency was calculated after 8 weeks of hardening.

Statistical analysis

The data variance (analysis of variance, ANOVA) was analyzed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA) for Windows. Significantly differing means were compared using Duncan's multiple-range test at 5% probability level.

Results and discussion

Embryogenic callus induction

After 8 weeks, 95.7% of the 282 cultured unopened female flower buds produced mostly hard calli. Besides the nonembryogenic callus, 4–10 SE structures were noticed on calli that had developed on the surface of the leaves of the flower buds (Fig. 1b) in 1.4% of the cultures. No somatic embryo structures were induced from cultured expanded flower buds or from vegetative bud explants (Table 1). Similarly, various types of leaf explants cultured on induction medium had >90% callus induction frequency, but only leaf explants from unopened flower buds induced somatic embryo structures (Fig. 1c). A low induction frequency of somatic embryo structures occurred on calli formed from veins at the basal part of the leaf explant incision site (Table 1).

Primary somatic embryo structures were soft, smooth, and easily separated from the callus up to a certain stage of development. Although they failed to germinate, most (92%) of the primary somatic embryo structures developed white translucent embryogenic calli within 12 weeks when cultured on medium containing 4.0 mg l^{-1} 2,4-D (Fig. 1d). The embryogenic calli initially proliferated slowly. After two or three subcultures, the proliferation rate increased, and the fresh weight increased more than 10-fold in 4 weeks. The overall proliferation frequency of embryogenic calli from flower buds was slower than that of zygotic embryos (Chen et al. 2010).

Floral structures and inflorescences have high potential for vegetative reproduction of several tree species (Gingas 1991; Lopez-Baez et al. 1993; Merkle et al. 1997; Steinmacher et al. 2007), possibly because reproductive tissues are more amenable to embryo induction than are vegetative tissues (Bonga et al. 2010). In our study, somatic embryos were induced only from flower bud leaves and not from vegetative bud leaves. It is uncertain why the two types of leaf explants responded differently to the induction signals. The competence for embryogenic induction of flower bud leaves may be the result of their proximity to rejuvenating sexual cells, as described by Bonga et al. (2010). Alternatively, endogenous hormone levels may be different in the two types of leaves. Endogenous hormone levels are crucial factors for determining the embryogenic potential of explants (Jiménez 2005). Both young and mature leaves are capable of indole-3-acetic acid (IAA) biosynthesis (Jager et al. 2007). Different leaf regions have higher IAA levels in embryogenic explants as compared with nonembryogenic ones

Table 1 Effect of female flower bud development stage and explant type on induction of somatic embryo structures of *S. chinensis*

Explant		Number of explants, cultured	Number of explants, induced SE	SE induction frequency (%)
Closed flower bud	Intact bud	282	4	1.4
Leaf bud	Basal part of leaf	420	4	1.0
	Apical part of leaf	396	0	0
Opened flower bud	Intact bud	324	0	0
Leaf bud	Basal part of leaf	440	0	0
	Apical part of leaf	410	0	0

Explants were subcultured after 4 weeks

Table 2 Effect of GA_3 concentration and duration of GA_3 treatment on germination and plantlet conversion of *S. chinensis* somatic embryos

GA_3 concentration (mg l^{-1})	Duration of GA_3 treatment (days)	Germination frequency (%)[a]	Plantlet conversion frequency (%)
0	0	34.0^{f}	19.4 ^{cd}
1	1	46.2^{bcd}	22.1^{c}
5	1	51.0^{b}	33.9b^{c}
10	1	27.6^{ef}	24.8^{c}
1	5	46.1b^{cd}	47.8^{a}
5	5	34.2^{cdef}	43.8^{ab}
10	5	23.3^{f}	49.2^{a}
1	10	31.0^{def}	56.4^{a}
5	10	42.3^{bcde}	21.4^{c}
10	10	28.3^{ef}	13.3 ^{cd}
1	15	78.5^{a}	23.7^{c}
5	15	47.3^{bc}	29.4^{bc}
10	15	36.8^{cdef}	2.7^{d}
GA_3 concentration (A)		***	**
Duration of GA_3 treatment (B)		***	***
A × B		**	***

[a] Average of 65 early-stage somatic embryos cultured in each flask. ** and ***, significant at $P < 0.01$ and 0.001, respectively (two-way ANOVA). For each variable, values followed by the same letter are not significantly different at $P < 0.05$

(Jiménez 2005). Based on field observations and our hydroponic cultivation experiments, dormant *S. chinensis* flower buds initiated development about 5 days earlier than did vegetative buds (data not shown). Hence, flower bud leaves may have higher embryogenic potential because they are more sensitive and respond earlier to IAA biosynthesis than do vegetative leaves under the same environmental conditions. In this study, only young unopened flower buds could induce somatic embryos. Similar responses have been described for other species. In sweetgum, young inflorescences were more competent for somatic embryo induction than more mature inflorescences (Merkle et al. 1998). In *Euterpe edulis*, somatic embryos were induced only when inflorescences at the early developmental stage were used (Guerra and Handro 1998).

Establishment of recurrent SE

In addition to the difficulty of SE induction, the appearance of abnormal embryos and the subsequently low frequency of plant conversion are severe constraints preventing practical application (Stasolla and Yeung 2003). Secondary SE is a process in which new somatic embryos are initiated from primary ones. This process has certain advantages compared with primary SE, such as independence from explants, ease of maintenance, high induction frequency, and repeatability (Dai et al. 2010). Moreover, secondary SE frequently occurs on medium lacking plant growth regulators (PGRs) (Nair and Gupta 2006; Yang et al. 2010b). For many plant species, the efficiency of plant conversion is higher with secondary embryos than with primary embryos (Raemakers et al. 1995).

Many independent globular embryos formed within 10 days and rapidly developed after embryogenic calli were transferred to 1/3-strength MS medium without PGRs (Fig. 1e). Ultimately, 53–76 mature somatic embryos formed from 5 mg embryogenic calli within 4 weeks (Fig. 1f), 34.0% of which germinated. Most germinated somatic embryos (83%) had abnormal trumpet-shaped embryos, and only 19% converted to plantlets (Fig. 1g). Both GA_3 concentration and the duration of GA_3 treatment significantly affected somatic embryo germination and conversion into plantlets. The highest frequency of germination and of plantlet conversion was obtained when somatic embryos were cultured in medium containing 1.0 mg l^{-1} GA_3 for 15 and 10 days, respectively. Both the germination and conversion frequencies were significantly affected by interactions between these two factors (Table 2). A significant stimulatory effect of GA_3 on somatic embryo germination and conversion into plantlets occurred in cultures of *Eschscholzia californica* (Park and Facchini 1999) and *Eleutherococcus senticosus* (Choi et al. 1999). In our study, GA_3 treatment increased the germination and plantlet conversion frequencies of somatic embryos when the optimal concentration and duration of treatment were used. Overall plantlet conversion frequency of mature somatic embryos was, however, lower than 20% for all treatments, suggesting that the somatic embryos were of low quality, as indicated by the presence of trumpet-shaped embryos.

Some germinated somatic embryos formed friable embryogenic calli on medium without PGRs. Medium with 1.0 or 4.0 mg l^{-1} 2,4-D significantly increased embryogenic callus induction. The proliferation of embryogenic

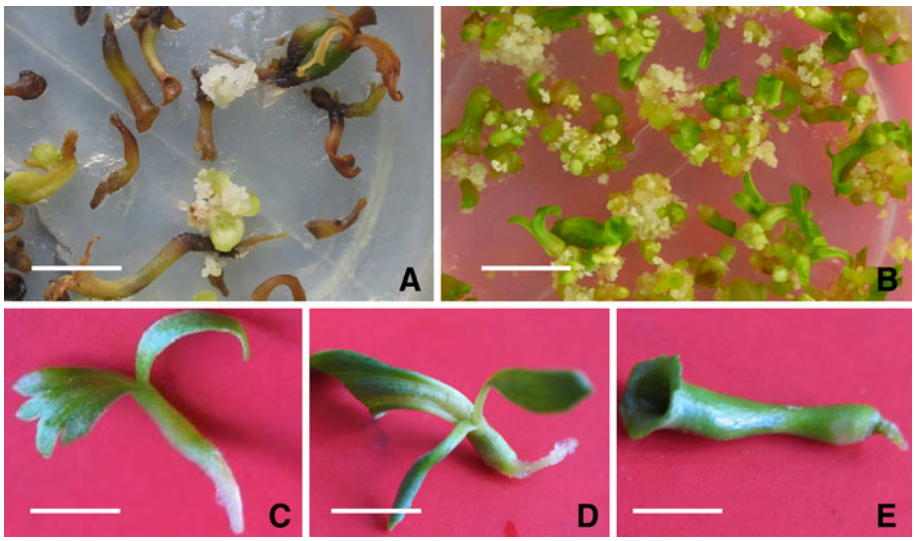

Fig. 2 Secondary SE from germinated somatic embryos of normal and abnormal somatic embryos. **a** Friable embryogenic calli induced from germinated somatic embryos after culturing on 1/3-strength MS medium containing 2% sucrose without PGRs for 8 weeks. *Bar* 6 mm. **b** Numerous somatic embryos developed after embryogenic calli were cultured in 1/3-strength MS medium containing 2% sucrose without PGRs for 12 weeks. *Bar* 4 mm. **c** Germinated somatic embryo with two cotyledons. *Bar* 3 mm. **d** Germinated somatic embryo with three cotyledons. *Bar* 3 mm. **e** Germinated somatic embryo with trumpet-shaped embryos. *Bar* 3 mm

calli and somatic embryo development were similar to the first cycle of secondary somatic embryos (data not shown), with most germinated somatic embryos having trumpet-shaped embryos. Friable embryogenic calli were induced at low frequency mostly from hypocotyl regions when cultured on PGR-free medium and were productive for about 3 weeks (Fig. 2a). When transferred to PGR-free medium, many somatic embryos formed asynchronously and quickly matured compared with medium containing 2,4-D within 4 weeks (Fig. 2b). The number of mature somatic embryos was slightly lower than that formed from embryogenic calli induced on medium containing 2,4-D.

Diverse somatic embryo types were observed in embryonic cultures induced in PGR-free medium (Fig. 2b), including somatic embryos with normally developed twin cotyledons (Fig. 2c), abnormal (three) cotyledons (Fig. 2d), and trumpet-shaped embryos (Fig. 2e). Germinated embryos had either two (~70%), three (~10%), or trumpet-shaped embryos (~20%). Microscopic observation showed that no proper apical meristems had formed in the barrel-shaped cotyledons. Histological observations indicated that all two- and three-cotyledon somatic embryos had defined shoot primordia, but most of the barrel-shaped somatic embryos lacked, or had only poorly developed, meristem tissue (data not shown). Plantlet conversion frequencies were 72.6%, 58.1%, and 10.2% for embryos containing two, three, and trumpet-shaped embryos, respectively. Germination and conversion frequencies of somatic embryos from embryonic calli grown on PGR-free medium reached 83.4% and 74.3%, respectively. Both were higher than from embryogenic calli induced on medium containing 2,4-D.

2,4-D is the most important PGR for initiation of somatic embryos (Jiménez 2005), although some evidence suggests that 2,4-D promotes the development of abnormal cotyledons, resulting in arrested plant conversion (Rodriguez and Wetzstein 1998; Hussain et al. 2009). In this study, a higher frequency of somatic embryos with trumpet-shaped embryos was observed in 2,4-D-induced cultures as compared with that in medium without PGRs. Histological analysis revealed that abnormal cotyledons lacked shoot apical meristems, which likely prevented conversion into plantlets even though the germination frequency was high. Pecan somatic embryos induced on medium containing 2,4-D also exhibit abnormalities and have a poorly developed shoot apical meristem (Rodriguez and Wetzstein 1998). Liu et al. (1993) suggested that auxin is synthesized in the shoot apical meristem and polar auxin transport is critical for regulating normal cotyledon formation, and hence disruption of polar auxin transport may cause abnormal somatic embryo cotyledon development. Therefore, elimination of 2,4-D during the culture process, even from the initiation medium, may be helpful for normal somatic embryo development and conversion into plantlets.

Fifty plantlets with well-developed root systems (Fig. 3a) were transferred to small containers containing autoclaved sand and soil. The regenerated plants were transplanted to soil, and about 90% developed normally (Fig. 3b).

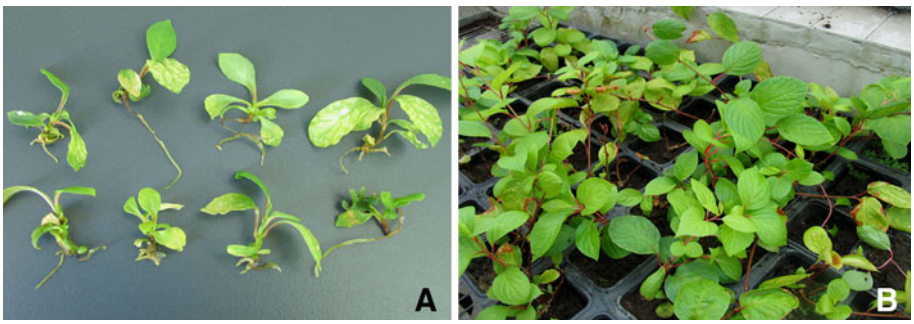

Fig. 3 Acclimatization of somatic embryo-derived plantlets. **a** Plantlets derived from somatic embryos. **b** One-year-old somatic plants grown in sand/soil mixture

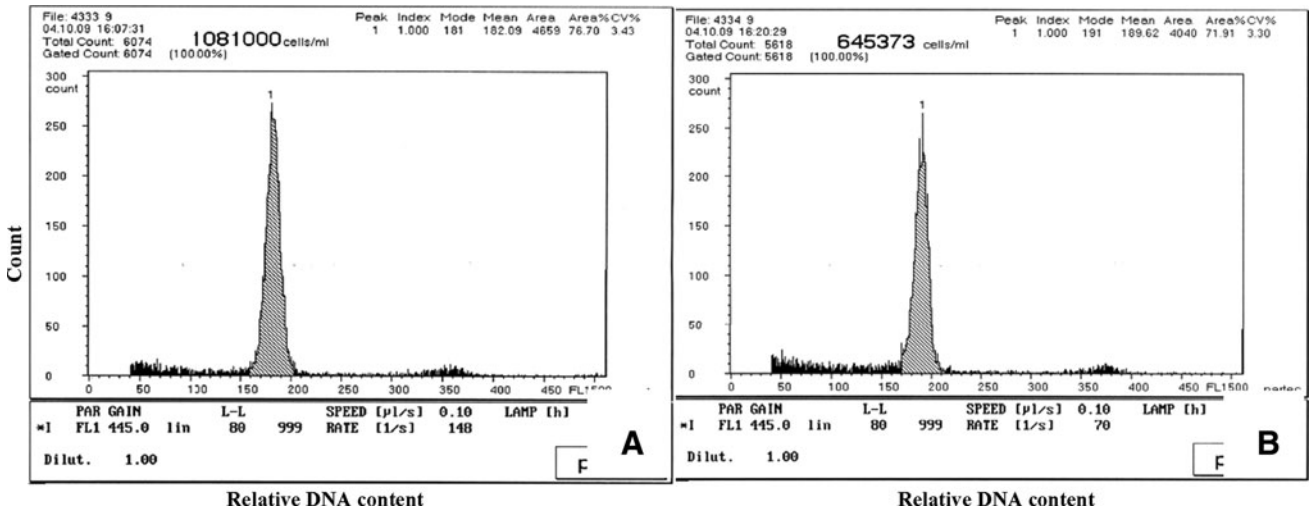

Fig. 4 Flow cytometry analysis of nuclei isolated from *S. chinensis*. **a** Relative fluorescence intensity generated from DNA isolated from leaves of somatic embryo-derived plantlets. **b** Relative fluorescence intensity generated from DNA isolated from leaves from a cutting of propagated donor plants

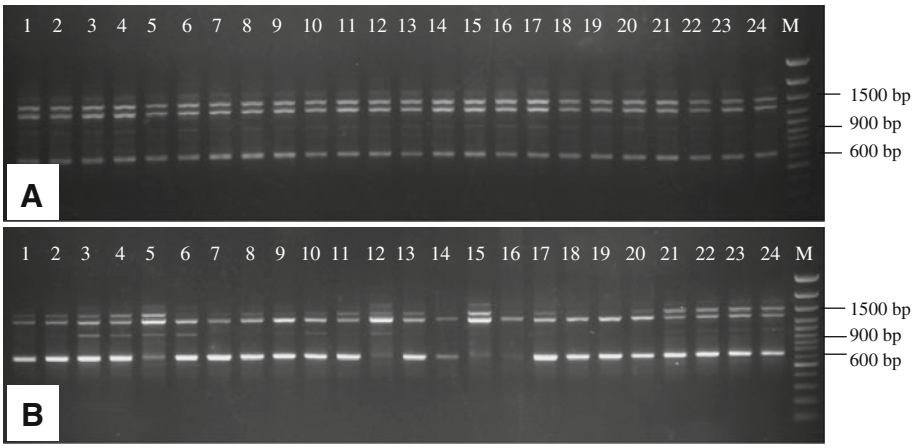

Fig. 5 RAPD analysis of *S. chinensis* polymorphisms using the OPA-17 primer pair. **a** *M*: 100 bp DNA ladder, *lanes* 1–20: seed-propagated plants, *lanes* 21–24: somatic embryo-derived plants. **b** *M*: 100 bp DNA ladder, *lanes* 1–20: somatic embryo-derived plants, *lanes* 21–24: cutting from propagated donor trees

Ploidy analysis

The ploidy of germinated somatic embryos and regenerated plants was the same. Also, the plants generated in the current study (Fig. 4a) had the same ploidy level (2*n*) as control donor trees (Fig. 4b).

Clonal identity analysis by RAPD

DNA was obtained from 20 somatic seedlings and amplified by PCR using 55 different primers. Two primers, OPA-17 (GACCGCTTGT) and OPJ-06 (TCGTTCCGCA), produced distinct bands, were highly polymorphic, and

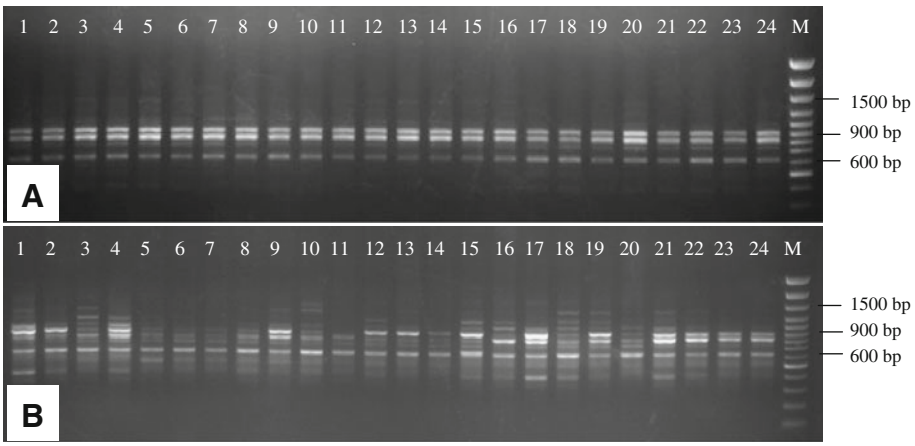

Fig. 6 RAPD analysis of *S. chinensis* polymorphisms using the OPJ-06 primer pair. **a** *M*: 100 bp DNA ladder, *lanes* 1–20: seed-propagated plants, *lanes* 21–24: somatic embryo-derived plants. **b** *M*: 100 bp DNA ladder, *lanes* 1–20: somatic embryo-derived plants, *lanes* 21–24: cutting from propagated donor trees

showed optimal reproducibility of the PCR products. Primer pairs OPA-17 and OPJ-06 were used for RAPD analysis to identify polymorphisms of randomly selected somatic embryo-derived plantlets. The results showed no differences between the plantlets from somatic embryos and parent plants (Figs. 5a, 6a). Plants regenerated from seed-propagated seedlings and somatic embryo-derived plantlets were, however, different and showed a larger variance in electrophoretic bands (Figs. 5b, 6b). Seed-propagated seedlings produced many irregular bands by primer pairs OPA-17 and OPJ-06, while only three regular equal bands were produced from SE-derived plantlets by the same primer pairs (Figs. 5, 6). The results indicated that somatic embryo-derived plants had higher genetic stability than did seed-propagated plants.

In conclusion, we have developed a cyclic somatic embryogenesis protocol for *S. chinensis* using flower bud explants from elite plants. The protocol established in this study will likely improve true-to-type clonal propagation of selected elite *S. chinensis*.

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References

Bonga JM, Klimaszewska KK, von Aderkas P (2010) Recalcitrance in clonal propagation, in particular of conifers. Plant Cell Tiss Org Cult 100:241–254

Chen AH, Yang JL, Niu YD, Yang CP, Liu GF, Li CH (2010) High-frequency somatic embryogenesis from germinated zygotic embryos of *Schisandra chinensis* and evaluation of the effects of medium strength, sucrose, GA_3, and BA on somatic embryo development. Plant Cell Tiss Org Cult 102:357–364

Choi YE, Kim JW, Yoon ES (1999) High frequency of plant production via somatic embryogenesis from callus or cell suspension cultures in *Eleutherococcus senticosus*. Ann Bot 83:309–314

Dai JL, Zhan YG, Zhang YQ, Xiao S, Gao Y, Xu DW, Wang T, Wang XC, You XL (2010) Rapid and repetitive plant regeneration of *Aralia elata* Seem. via somatic embryogenesis. Plant Cell Tiss Org Cult 104:125–130

Gingas VM (1991) A sexual embryogenesis and plant regeneration from female catkins of *Quercus*. Hortscience 26:1217–1218

Guerra MP, Handro W (1998) Somatic embriogenesis and plant regeneration in different organs of *Euterpe edulis* Mart. (Palmae): control and structural features. J Plant Res 111:65–71

Hancke JL, Burgos RA, Ahumada F (1999) *Schisandra chinensis* (Turcz.) Baill. Fitoterapia 70:451–471

Hussain SS, Rao AQ, Husnain T, Riazuddin S (2009) Cotton somatic embryo morphology affects its conversion to plant. Biol Plant 53:307–311

Jager C, Symons GM, Glancy NE, Reid JB, Ross JJ (2007) Evidence that the mature leaves contribute auxin to the immature tissues of pea (*Pisum sativum* L.). Planta 226:361–368

Jiménez VM (2005) Involvement of plant hormones and plant growth regulators on in vitro somatic embryogenesis. Plant Growth Regul 47:91–110

Kim TD, Anbazhagan VR, Park JI (2005) Somatic embryogenesis in *Schisandra chinensis* (Turcz.) BAILL. In Vitro Cell Dev Biol Plant 41:253–257

Liu CM, Xu ZH, Chua NH (1993) Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. Plant Cell 5:621–630

Lopez-Baez O, Bollon H, Eskes A, Petiard V (1993) Somatic embryogenesis and plant regeneration from flower parts of cocoa *Theobroma cacao* L. C R Acad Sci Paris 316:579–584

Mallón R, Rodríguez-Oubiña J, González ML (2010) In vitro propagation of the endangered plant *Centaurea ultreiae*: assessment of genetic stability by cytological studies, flow cytometry and RAPD analysis. Plant Cell Tiss Org Cult 101:31–39

Merkle SA, Bailey RL, Pauley BA, Neu KA, Kim MK, Rugh CL, Montello PM (1997) Somatic embryogenesis from tissues of mature sweetgum trees. Can J For Res 27:959–964

Merkle SA, Neu KA, Battle PJ, Bailey RL (1998) Somatic embryogenesis and plantlet regeneration from immature and mature tissues of sweetgum (*Liquidambar styraciflua)*. Plant Sci 132:169–178

Mishra J, Singh M, Palni LMS, Nandi SK (2011) Assessment of genetic fidelity of encapsulated microshoots of *Picrorhiza kurrooa*. Plant Cell Tiss Org Cult 104:181–186

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Murray MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Nair RR, Gupta SD (2006) High-frequency plant regeneration through cyclic secondary somatic embryogenesis in black pepper (*Piper nigrum* L). Plant Cell Rep 24:699–707

Park SU, Facchini PJ (1999) High-efficiency somatic embryogenesis and plant regeneration in California poppy, *Eschscholzia californica* Cham. Plant Cell Rep 19:421–426

Raemakers CJJM, Jacobsen E, Visser RGF (1995) Secondary somatic embryogenesis and applications in plant breeding. Euphytica 81:93–107

Rodriguez APM, Wetzstein HY (1998) A morphological and histological comparison of the initiation and development of Pecan (Carya illinoinensis) somatic embryogenic cultures induced with naphthaleneacetic acid or 2, 4-dichlorophenoxyacetic acid. Protoplasma 204:71–83

Smiskova A, Vlasinova H, Havel L (2005) Somatic embryogenesis from zygotic embryos of *Schisandra chinensis*. Biol Plant 49:451–454

Stasolla C, Yeung EC (2003) Recent advances in conifer somatic embryogenesis: improving somatic embryo quality. Plant Cell Tiss Org Cult 74:15–35

Steinmacher DA, Clement CR, Guerra MP (2007) Somatic embryogenesis from immature peach palm inflorescence explants: towards development af an efficient protocol. Plant Cell Tiss Org Cult 89:15–22

Tang W (2001) In vitro regeneration of loblolly pine and random amplified polymorphic DNA analysis of regenerated plantlets. Plant Cell Rep 20:163–168

Tremblay L, Levasseur C, Tremblay FM (1999) Frequency of somaclonal variation in plants of black spruce (*Picea mariana*, Pinaceae) and white spruce (*P. glauca*, Pinaceae) derived from somatic embryogenesis and identification of some factors involved in genetic instability. Am J Bot 86:1373–1381

Yang JL, Seong ES, Kim MJ, Ghimire BK, Kang WH, Yu CY, Li CH (2010a) Direct somatic embryogenesis from pericycle cells of broccoli (*Brassica oleracea* L. var. italica) root explants. Plant Cell Tiss Org Cult 100:49–58

Yang JL, Zhao B, Seong ES, Kim MJ, Kang WH, Kim NY, Yu CY, Li CH (2010b) Callus induction and high-efficiency plant regeneration via somatic embryogenesis in *Papaver nudicaule* L., an ornamental medicinal plant. Plant Biotechnol Rep 4:261–267