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# SOMATIC EMBRYOGENESIS AND PLANT REGENERATION FROM IMMATURE EMBRYO COTYLEDONS OF *SYRINGA RETICULATA* BLUME VAR. *MANDSHURICA* HARA

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**Abstract**

A somatic embryogenesis and plant regeneration protocol was established for *Syringa reticulata* Blume var. *mandshurica* Hara from immature embryo cotyledon explants. Somatic embryos were induced on half-strength Murashige and Skoog medium supplemented with 400 mg $l^{-1}$ casein hydrolysate (CH) and different concentrations of sucrose (30, 50 and 70 g $l^{-1}$), NAA (0, 5.37, 8.05 and 10.74 μM), and BA (0 and 2.22 μM). Fifty-four percent of explants produced somatic embryos and seven somatic embryos were induced per explant on medium supplemented with 70 g $l^{-1}$ sucrose, 10.74 μM NAA and 2.22 μM BA. Over 80% of mature somatic embryos germinated on half strength MS medium supplemented with 50 g $l^{-1}$ sucrose and 0.05 μM NAA. Five of ten regenerated plantlets survived after 60 days of transplantation and acclimatization in greenhouse. Histological observation of somatic embryos showed similar developmental processes to those of zygotic embryos, with globular, heart, torpedo, and cotyledon stages. Moreover, the gradual disappearance of the suspensor was also observed after the late-globular stage; the suspensor disappeared completely after the heart stage.

**Key words:** Explant browning, immature zygotic embryos, manchurian lilac, somatic embryo induction



## INTRODUCTION

Species of the genus *Syringa* are renowned as excellent ornamentals for landscaping due to their colorful flowers and fragrant scent. Manchurian lilac (*Syringa reticulata* Blume var. *mandshurica* Hara) have been used for ornamentals in China especially in Northeast China extensively for its beautiful flowers and strong fragrant (Lu 2010, Zhao 2011). Seed propagation has always been the main approach for *S. reticulata* propagation (Zhao 2011, Yan et al. 2010), but the recent scarcity of seeds has limited seed propagation. Some researchers have focused on *in vitro* culture for this genus (Refouvelet et al. 1998, Hilderbrandt and Harney 1983, Gabryszewska 1989, Pierik et al. 1988, Waldenmaier and Bünemann 1992, Zhou et al. 2003a,b, Liu et al. 1995), and Liu et al. (2003a,b, 2004) induced adventitious shoots and callus using hypocotyls of Manchurian lilac as explants, yet were unable to induce somatic embryos. Considering its advantages in producing regenerated plantlets in large quantities (Rout 1991) and its potential for mass propagation of elite genotypes (Bonga et al. 2000, Menéndez-Yuffá et al. 2010, Parimalan et al. 2011), as well as its contributions to genetic engineering (Kumar et al. 2006, 2007) and artificial seed production (Maruyama et al. 2003), somatic embryogenesis (SE) offers the optimal method for commercial cloning of genetically improved genotypes, which is especially crucial for ornamental species (Attree et al. 1992). However, a review of the research on *in vitro* culture of *Syringa* suggests that somatic embryos have not yet been induced successfully. In this study, we describe a protocol for regeneration of *S. reticulata* via SE, in which immature embryo cotyledons were used as explants and surviving plantlets were obtained successfully in a greenhouse. This is the first report of SE and plant regeneration in the genus *Syringa*. Our method based on SE offers an efficient pathway for large-scale propagation of natural resources, especially the elite genotypes of *S. reticulata* for use as garden ornamentals. In addition, the protocol might be applicable to SE of other species in genus *Syringa*.

## MATERIALS AND METHODS

### *Plant material collection*

Immature fruits of Manchurian lilac were gathered on July 28, 2007 in the Northeast Forestry University

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Forest in Harbin. The fruits were washed for 1 day under running water and then disinfected in 10% $H_2O_2$ (v/v) for 15 min, immersed in 70% ethanol for 30 s, and rinsed five times with sterile water. One third (approximately 5 mm in length) of the fruits on the side of hypocotyls was excised using a scalpel, and the immature cotyledons were pressed out using forceps. Single cotyledons were then placed onto induction media (the inside of the cotyledon closest to the media). All operations after disinfection were performed on a clean bench.

### *Induction media*

Young cotyledons were placed on half strength MS medium (all components are half-strength of MS medium) (Kong et al. 2012) supplemented with 400 mg $l^{-1}$ casein hydrolysate (CH; Sigma-Aldrich Co. Ltd., Shanghai, China), 6 g $l^{-1}$ agar (Junsei Chemical Co. Ltd., Tokyo, Japan) and different concentrations of sucrose (30, 50, 70 g $l^{-1}$), BA (0, 2.22 μM; Sigma-Aldrich Co. Ltd.) and NAA (0, 5.37, 8.05, 10.74 μM; Sigma-Aldrich Co. Ltd.). All media were adjusted to pH 5.8 with 0.1 N NaOH, and then autoclaved at 121°C for 20 min. Twenty-four treatments were arranged according to a completely random design. Each treatment was replicated five times and each replicate comprised 10 explants in a 90-mm Petri dish. The cultures were transferred onto fresh media of the same composition after 30 days of induction culture. After 45 days of induction culture, the number of somatic embryos per explant was recorded, and the induction rates of callus and somatic embryos (including indirect SE, direct SE and total SE) were calculated as follows:

- Callus induction rate (%) = Explants producing calluses / Total explants × 100;
- Indirect somatic embryo induction rate (%) = Explants producing somatic embryos from calluses / Total explants × 100;
- Direct somatic embryo induction rate (%) = Explants producing somatic embryos directly / Total explants × 100;
- Total somatic embryo induction rate (%) = Indirect somatic embryo induction rate (%) + Direct somatic embryo induction rate (%).

### *Maturation and germination media*

Half strength MS media supplemented with 70 g $l^{-1}$ sucrose and 6 g $l^{-1}$ agar were used for maturation of the somatic embryos. The mature somatic embryos were then incubated on ½ MS media supplemented with 50 g $l^{-1}$ sucrose, 0.05 μM NAA and 6 g $l^{-1}$ agar for germination. All media were adjusted to pH 5.8 with 0.1 N NaOH, and then autoclaved at 121°C for 20 min.

### *Culture conditions*

Induction and maturation cultures were performed in darkness, and germination cultures were placed in the light with a 16 h photoperiod at a light intensity of 2400 lux. Both dark and light cultures were maintained in a growth chamber with relative humidity 40-70% at 24 ± 2°C.

### *Histological observation*

The cultures were observed periodically and the morphogenesis of the embryos was examined using a stereoscopic microscope (Olympus SZX–ILLB2-200, Olympus, Tokyo, Japan). Samples in different developmental stages were fixed in FAA (5 formalin : 5 glacial acetic acid : 90 50% ethanol, v/v/v). The samples were then placed in 50% (v/v) ethanol for 1 h to eliminate FAA before staining with hematoxylin–eosin (Sigma-Aldrich Co. Ltd.). Microexaminations were performed to check the staining process. The stained samples were dehydrated through a gradient ethanol series, and then through dimethylbenzene and gradually embedded in paraffin (Melting point 52-54°C). Embedded tissues were sliced into 8-μm thick sections and then observed under a microscope (Olympus BX51, Olympus) with a digital camera attached (Moticam 3000C, Motic, Japan).

### *Statistical analysis*

All percentage data were converted into decimals for analysis. ANOVA was performed using SPSS 13.0 to analyze the experimental data and the means were tested with an LSD multiple range test ($\alpha = 0.05$). The data were recorded as means ± SD of five replicates.

## RESULTS

### *Induction of somatic embryogenesis*

Significant differences in the induction rates of callus and somatic embryos (including indirect, direct and total somatic embryos), and the number of somatic embryos per explant were found among the 24 treatments ($p < 0.05$, Table 1). The somatic embryo induction rate and the number of somatic embryos per explant ranged from 0-54% and 0-7, respectively, and both the highest somatic embryo induction rate (54%, including indirect and direct somatic embryo induction) and number of somatic embryos (6.7) were found in the treatment supplemented with 70 g $l^{-1}$ sucrose, 2.22 μM BA and 10.74 μM NAA. However, the mean somatic embryo induction rate among the 24 treatments was only 14.7% (Table 1), of which indirect SE induction comprised 5% and direct SE induction comprised 10% (Table 1). The callus induction rate reached 84% when explants were incubated on media supplemented with 2.22 μM BA and 8.05 μM NAA. Under this combination of NAA and BA, sucrose concentration had no significant effect

**Table 1. Induction of somatic embryos and callus from young cotyledons of *Syringa reticulata* on different culture media.**

| Sucrose (g $l^{-1}$) | BA (μM) | NAA (μM) | Callus induction rate (%)a | Somatic embryo induction rate (%)[a] | | | No. somatic embryos |
|---|---|---|---|---|---|---|---|
| | | | | Indirect | Direct | Total | |
| 30 | 0 | 0.00 | 0.0 ± 0.0 g | 0.0 ± 0.0 e | 0.0 ± 0.0 e | 0.0 ± 0.0 f | 0.0 ± 0.0 e |
| | | 5.37 | 28.0 ± 17.9 cdef | 0.0 ± 0.0 e | 2.0 ± 4.5 de | 2.0 ± 4.5 de | 0.2 ± 0.3 e |
| | | 8.05 | 32.0 ± 13.0 cde | 2.0 ± 4.5 e | 0.0 ± 0.0 e | 2.0 ± 4.5 de | 0.1 ± 0.2 e |
| | | 10.74 | 46.0 ± 41.6 bc | 4.0 ± 8.9 de | 0.0 ± 0.0 e | 4.0 ± 8.9 de | 0.3 ± 0.5 de |
| | 2.22 | 0.00 | 4.0 ± 5.5 fg | 0.0 ± 0.0 e | 6.0 ± 8.9 cde | 6.0 ± 8.9 cde | 0.1 ± 0.1 e |
| | | 5.37 | 38.0 ± 48.2 cd | 8.0 ± 17.9 cde | 10.0 ± 10.0 cde | 18.0 ± 14.8 cde | 0.8 ± 0.8 cde |
| | | 8.05 | 84.0 ± 18.2 a | 6.0 ± 8.9 cde | 4.0 ± 8.9 cde | 10.0 ± 10.0 cde | 0.3 ± 0.4 de |
| | | 10.74 | 82.0 ± 34.9 a | 18.0 ± 13.0 ab | 8.0 ± 13.0 cde | 26.0 ± 11.4 bc | 1.1 ± 0.9 cde |
| 50 | 0 | 0.00 | 2.0 ± 4.5 g | 0.0 ± 0.0 e | 0.0 ± 0.0 e | 0.0 ± 0.0 e | 0.0 ± 0.0 e |
| | | 5.37 | 14.0 ± 15.2 defg | 0.0 ± 0.0 e | 6.0 ± 8.9 cde | 6.0 ± 8.9 cde | 0.3 ± 0.5 de |
| | | 8.05 | 34.0 ± 16.7 cde | 0.0 ± 0.0 e | 4.0 ± 8.9 cde | 4.0 ± 8.9 cde | 0.1 ± 0.2 e |
| | | 10.74 | 38.0 ± 14.8 cd | 2.0 ± 4.4 e | 8.0 ± 8.4 cde | 10.0 ± 12.2 ef | 0.4 ± 0.5 de |
| | 2.22 | 0.00 | 2.0 ± 17.3 defg | 0.0 ± 0.0 e | 10.0 ± 14.1 cde | 10.0 ± 14.1 ef | 0.3 ± 0.4 de |
| | | 5.37 | 72.0 ± 17.9 a | 14.0 ± 5.4 bc | 16.0 ± 16.7 bc | 30.0 ± 18.7 bc | 0.9 ± 0.4 cde |
| | | 8.05 | 84.0 ± 20.7 a | 6.0 ± 8.9 cde | 12.0 ± 17.9 cde | 18.0 ± 14.8 cde | 0.6 ± 0.4 cde |
| | | 10.74 | 68.0 ±17.9 ab | 14.0 ± 11.4 bc | 14.0 ± 16.7 bcd | 28.0 ± 10.9 bc | 1.0 ± 0.6 cde |
| 70 | 0 | 0.00 | 0.0 ± 0.0g | 0.0 ± 0.0 e | 4.0 ± 8.9 cde | 4.0 ± 8.9 f | 0.1 ± 0.2 e |
| | | 5.37 | 4.0 ± 5.5 fg | 0.0 ± 0.0 e | 0.0 ± 0.0 e | 0.0 ± 0.0 f | 0.0 ± 0.0 e |
| | | 8.05 | 10.0 ± 17.3 efg | 0.0 ± 0.0 e | 12.0 ± 13.0 cde | 12.0 ± 13.0 cde | 0.4 ± 0.5 de |
| | | 10.74 | 14.0 ± 8.9 defg | 0.0 ± 0.0 e | 6.0 ± 8.9 cde | 6.0 ± 8.9 cde | 0.3 ±0.4 de |
| | 2.22 | 0.00 | 18.0 ± 13.0 defg | 0.0 ± 0.0 e | 32.0 ± 4.5 a | 32.0 ± 4.5 a | 1.4 ± 0.4 bcd |
| | | 5.37 | 74.0 ± 11.4 a | 20.0 ± 12.2 ab | 26.0 ± 11.4 ab | 46.0 ± 11.4 a | 2.6 ±1.2 b |
| | | 8.05 | 84.0 ± 13.4 a | 12.0 ± .4 bcd | 12.0 ± 13.0 cde | 24.0 ± 11.4 bcd | 1.7 ± 0.6 bc |
| | | 10.74 | 68.0 ± 19.2 ab | 24.0 ± 11.4 a | 30.0 ± 15.8 a | 54.0 ± 18.2 a | 6.8 ± 4.2 a |
| Mean | | | 38.3 ± 35.2 | 5.4 ± 9.8 | 9.3 ± 13.0 | 14.7 ± 17.5 | 0.8 ± 1.6 |

Different lowercase letters indicate significant difference in an LSD multiple comparison test (α = 0.05).

on callus induction (Table 1).

Explants from the immature embryo cotyledons expanded, shrank and then formed protuberances on the cotyledon surfaces on the 5th day of culture (Fig. 1A). Yellow and white loose nonembryogenic calluses developed from the cut ends of the explants on the 13th day of culture (Fig. 1B). Somatic embryos of *S. reticulata* were produced both indirectly (from embryogenic calluses, Fig. 1C, D) and directly (from the explants, Fig. 1E). Similarly to zygotic embryos, somatic embryo development progressed through globular, heart, torpedo and cotyledon stages (Fig. 1F-I), which began after 3 weeks of culture; some somatic embryos were also found to be polycotyledonous (Fig. 1J). When the somatic embryos were incubated on the induction medium, some secondary somatic embryos developed from the primary embryos (Fig. 1K) and even from browned primary somatic embryos (Fig. 1L).

### *Effects of sucrose, BA and NAA on somatic embryogenesis*

To evaluate the effects of sucrose concentrations on SE, media supplemented with 2.22 μM BA and 10.74 μM NAA were used for analysis because of their high somatic embryo induction rates (Table 1). Similarly, media supplemented with 70 g $l^{-1}$ sucrose were used for analyzing the effects of BA and NAA concentrations on SE. Sucrose concentration significantly affected both somatic embryo induction rate and number of somatic embryos, but had no significant effect on callus induction ($p > 0.05$). A high concentration of sucrose (70 g $l^{-1}$) improved both somatic embryo induction rate and the number of somatic embryos significantly.

Both BA and NAA concentrations, as well as their interaction, affected callus induction rate, somatic embryo induction rate and number of somatic embryos significantly, except that NAA did not have a significant effect on SE ($p > 0.05$). A combination of auxin and cytokinins had greater effect on both callus formation and SE than auxin or cytokinins alone (Table 1). The highest induction rate and number of somatic embryos were found with a combination of 2.22 μM BA and 10.74 μM NAA (Table 1).

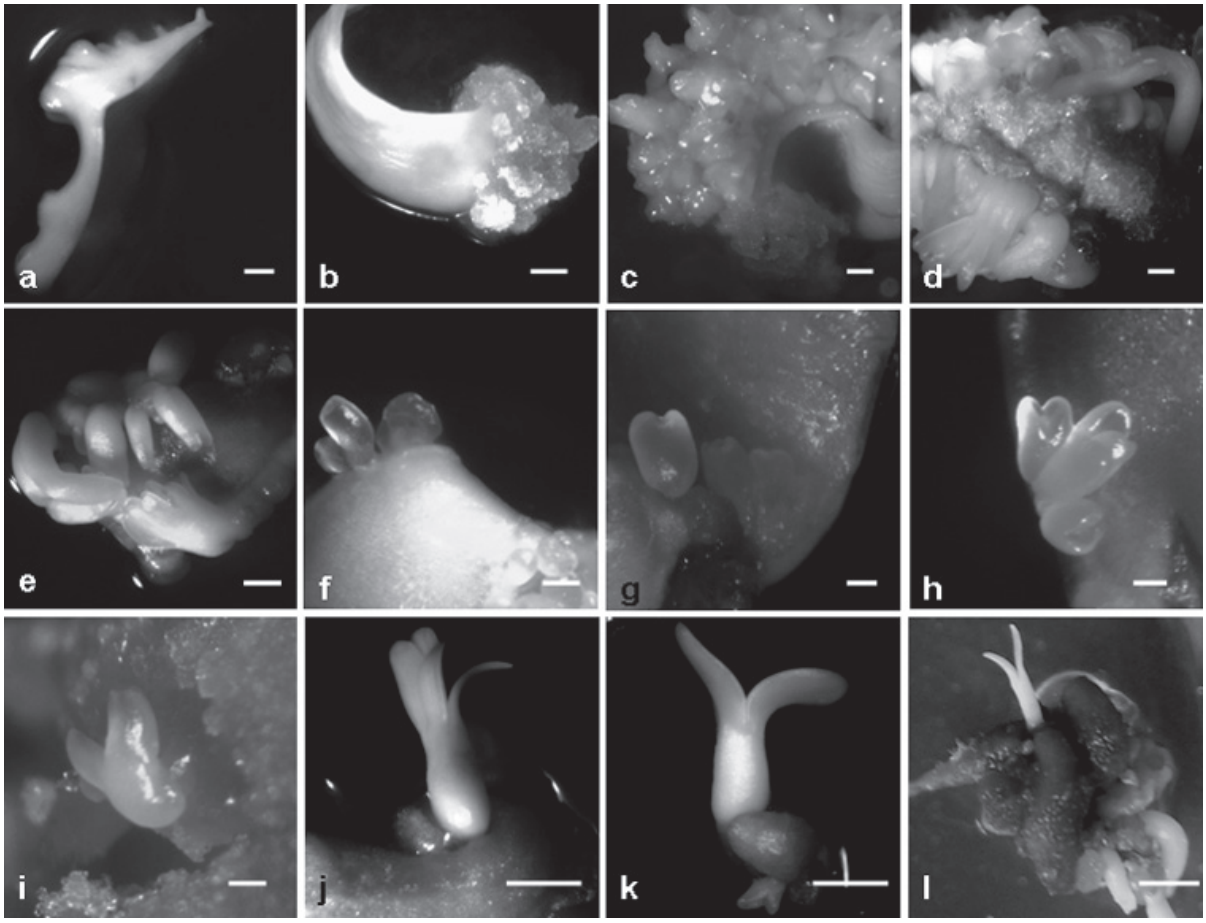


**Fig. 1.** Somatic embryogenesis and developmental stages of somatic embryos of *S. reticulata*. A) Prominences on the explant surfaces, B) Nonembryogenic calluses, C) Embryogenic calluses, D-E) Somatic embryos produced from calluses (D) and explants (E), F-I) Globular (F), heart (G), torpedo (H), and cotyledon (I) stage embryos, J) A polycotyledonous embryo, **K)** Secondary somatic embryos produced on somatic embryos, L) Secondary somatic embryos produced on browned somatic embryos. Scale bars = 1 mm (A-H), 2 mm (**I**) and 5 mm (J-L).

***Maturation and germination of the somatic embryos***

After the somatic embryos developed to the cotyledon stage, the cotyledonary somatic embryos were separated from the explants and placed on half strength MS medium supplemented with 50 g $l^{-1}$ sucrose and 6 g $l^{-1}$ agar for maturation. The somatic embryos that became an opaque milky or ivory white were considered to be mature. The mature embryos were then transferred to half strength MS medium supplemented with 50 g $l^{-1}$ sucrose, 0.05 µM NAA and 6 g $l^{-1}$ agar for germination.

Cotyledonary somatic embryos became green after 3 days incubation (Fig. 2A) and germinated fully after 3 weeks incubation on germination media (Fig. 2B); the germination rate of somatic embryos was 81.0%. The germinated somatic embryos grew on germination medium sequentially and then developed into complete plantlets with true leaves and roots (Fig. 2C). Some of the regenerated plantlets were abnormal, and appeared to be polycotyledonary (Fig. 2D, E). Almost all plantlets rooted on the germination media, thus a rooting culture was not needed before transplantation.

Ten plantlets were transplanted into 8-cm plastic pots with sterilized substrate (5 peat moss : 4 vermiculite : 1 perlite, v/v/v) after they formed more than three true leaves. The plantlets were placed in growth chamber and covered with plastic wrap for the first week of transplantation. The cover was then removed gradually during the next week, seven regenerated plantlets survived at this stage. After that, the plantlets were placed in a greenhouse; five of them survived after 30 days of acclimatization (Fig. 2G).

***Histological observations***

Somatic embryogenesis of Manchurian lilac showed two patterns: direct embryogenesis, where an embryogenic cell mass formed directly from internal explant cells (Fig. 3A) or epidermal cells (Fig. 3B); and indirect embryogenesis, in which embryos were formed from embryogenic calluses (Fig. 3C). Similar to zygotic embryogenesis, the development of somatic

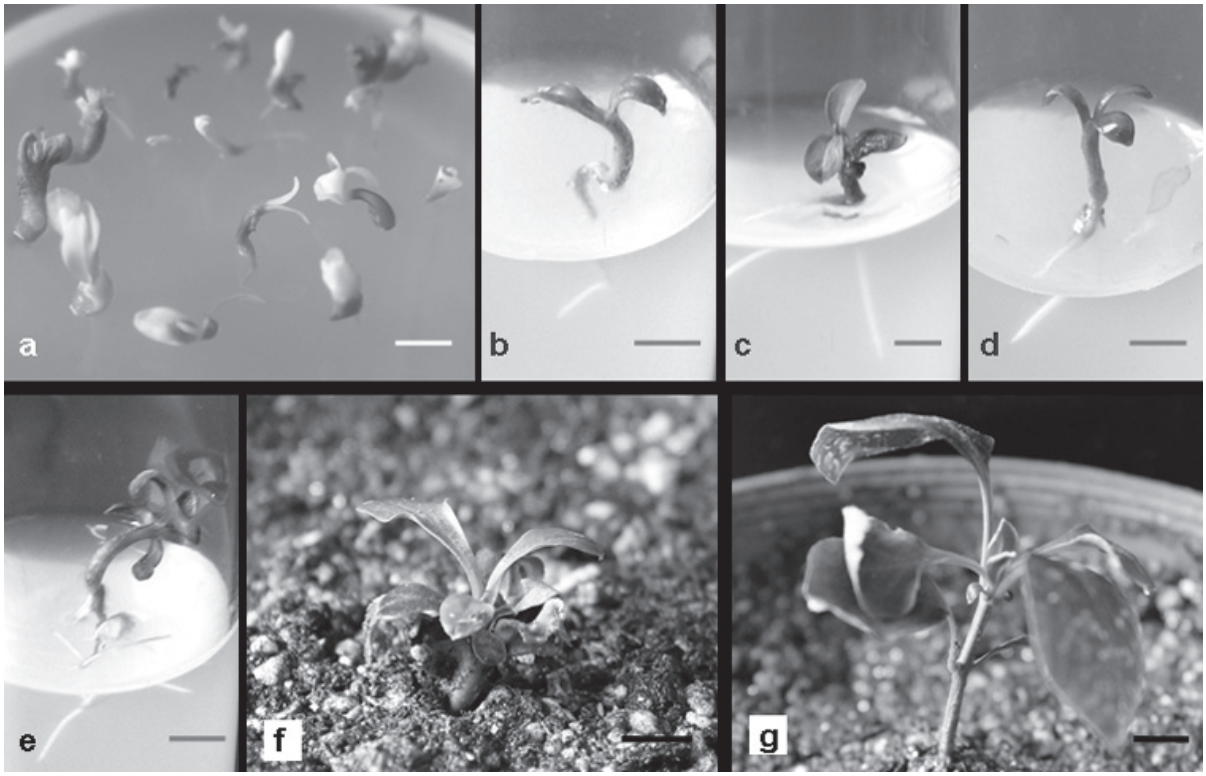


**Fig. 2.** Germination of somatic embryos and plantlet regeneration of *Syringa reticulata*. A) Germinating somatic embryos, B-C) Germinated somatic embryos with two cotyledons (B) and secondary embryos produced on browned abnormal somatic embryos (C), D-E) Abnormal polycotyledonous embryos with three (D) and four cotyledons (E), F) Regenerated plantlets 14 days after transplantation, G) Regenerated plantlets surviving after 30 days of acclimatization in a greenhouse. Scale bars = 5 mm (A), 10 mm (B-G).

embryos proceeded through globular (Fig. 3D), heart (Fig. 3E), torpedo (Fig. 3F) and cotyledon stages (Fig. 3G). A suspensor was present during the globular stage (Fig. 3B,C), but disappeared gradually during the late globular stage (Fig. 3D) and was completely gone in the heart stage (Fig. 3E-G). A vascular bundle was clearly observed when the somatic embryos developed to the cotyledon stage (Fig. 3G). In addition, secondary somatic embryos were observed on the surface of the primary somatic embryos (Fig. 3H).

## DISCUSSION

Some research in the past several decades has been focused on *in vitro* culture of genus *Syringa* (Refouvelet et al. 1998, Hilderbrandt and Harney 1983, Gabryszewska 1989, Pierik et al. 1988, Waldenmaier and Bünemann 1992, Zhou et al. 2003a, b, Liu et al. 1995, Liu and Shen 2003). However, to our knowledge, this is the first report to study SE of this genus. In this study, a preliminary plant regeneration system has been successfully established for *S. reticulata* via SE from young cotyledon explants. The preferable medium for SE was 1/2MS medium supplemented with 400 mg $l^{-1}$ CH, 70 g $l^{-1}$ sucrose, 10.74 µM NAA and 2.22 µM BA. Somatic embryos were matured on plant growth regulator (PGR)-free 1/2MS media supplemented with 70 g $l^{-1}$ sucrose and then germinated on half strength MS media supplemented with 50 g $l^{-1}$ sucrose and 0.05 µM NAA. The regenerated plantlets were transplanted to substrate containing 5 peat moss : 4 vermiculite : 1 perlite (v/v/v), and half of them survived after transplantation and acclimatization.

Somatic embryogenesis of *S. reticulata* showed two patterns: direct embryogenesis, where embryos are formed directly from explants; and indirect embryogenesis, in which callus formation precedes the embryogenesis process (Denchev et al. 1993). Direct somatic embryogenesis is preferred for obtaining genetically stable regenerated plants, because callus formation causes somaclonal variation (Larkin et al. 1981, Gaj 2001), which is often heritable, and therefore unwanted in somatic clones (Qin et al. 2007).

Histological observation captured the developmental process of somatic embryos of *S. reticulata* and confirmed its similarity to zygotic embryogenesis, as in many other species (Capelo et al. 2010, Kong et al. 2012, Yang et al. 2012), with globular, heart, torpedo and cotyledon stages accompanied by the disappearance

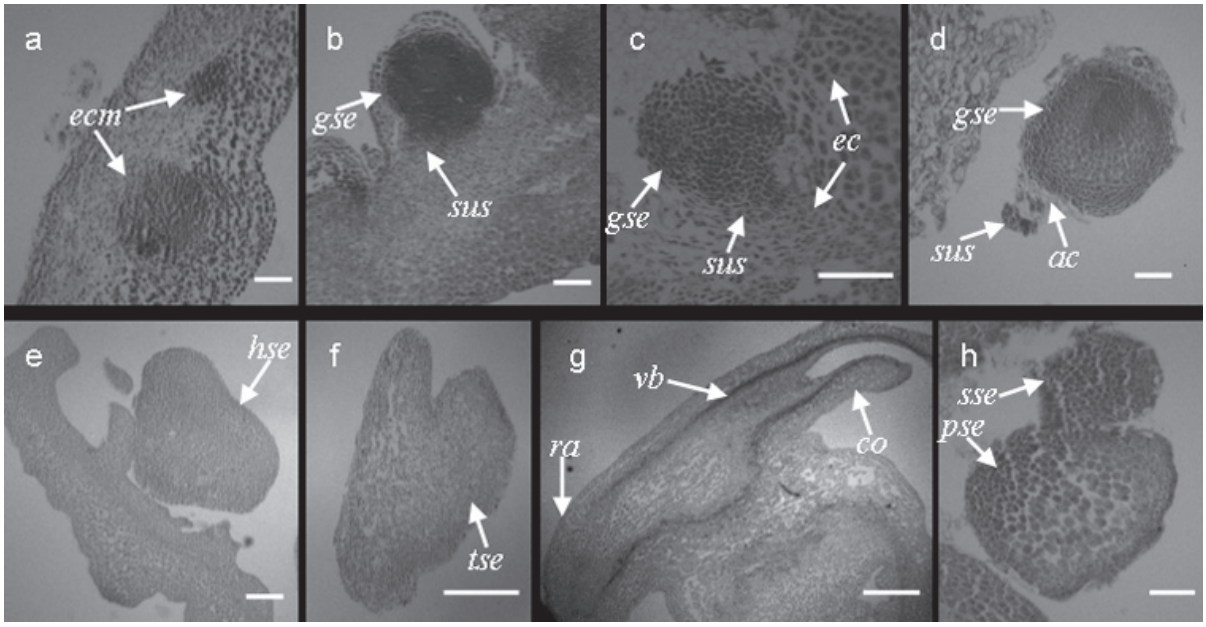


**Fig. 3.** Histological observation of somatic embryogenesis and the developmental stages of somatic embryos of *S. reticulata*. A-B) Somatic embryos formed directly from explant internal cells (A) and epidermal cells (B), C) Somatic embryos formed indirectly from embryogenesis calluses, D-G) Somatic embryos at the globular (D), heart (E), torpedo (F) and cotyledonary (G) stages, H) Secondary somatic embryos formed on primary somatic embryos. Legend: *ecm* - bembryogenic cell mass, *gse* - globular somatic embryo, *sus* - suspensor, *ec* - embryogenic callus, *ac* - apoptotic cells, *hse* - heart somatic embryo, *tse* - torpedo somatic embryo, *ra* - radicle, *vb* - vascular bundle, *co* - cotyledon, *pse* - primary somatic embryo, *sse* - secondary somatic embryo. Scale bars = 100 μm (A-D, H), 200 μm (E) and 500 μm (F,G).

of a suspensor during these processes.

PGRs, especially auxins and cytokinins, strongly influence the induction and control of somatic embryogenesis (Jiménez 2005, Pinto et al. 2008, Don Palmer and Keller 2011). In this study, a combination of auxin and cytokinins was better than using auxin or cytokinins alone for somatic embryo induction, which is in accordance with other research (Arunyanart and Chaitrayagun 2005, Karami et al. 2006, Shen et al. 2011). Usually, somatic embryos can germinate and develop into regenerated plantlets on medium without PGRs (Yang et al. 2012, Xu et al. 2012, Buendía-González et al. 2012). However, as in this study, a low concentration of PGRs such as NAA (Kong et al. 2012) and $GA_3$ (Sivanesan et al. 2011) is sometimes used for promoting the germination and conversion of somatic embryos.

Carbohydrates play a vital role in the plant life cycle and sucrose is the most common carbohydrate source used in plant tissue culture. The sugar concentration in the culture medium affects the formation of somatic embryos, but the concentration required for efficient SE varies by species. In some species, including *S. reticulata*, a high sucrose concentration such as 68.5 g $l^{-1}$ in *Cucumis melo* L. (Nakagawa et al. 2001), 70 g $l^{-1}$ in *Fraxinus mandshurica* Rupr. (Kong et al. 2012), 88.5 g $l^{-1}$ in *Cucumis sativus* L. (Lou and Kako, 1995) and 120 g $l^{-1}$ in *Dianthus caryophyllus* L. (Karami et al. 2006) is beneficial to SE. In other species, a high sucrose concentration can inhibit SE. For example, 60 g $l^{-1}$ sucrose reduced somatic embryo induction in *Sorbus phohuashanensis* Hedl. compared with 40 g $l^{-1}$, and when the sucrose concentration was at 80 or 100 g $l^{-1}$, no somatic embryos were induced (Yang et al. 2012). Similarly, in *Aralia elata* Seem., secondary SE was reduced significantly when the sucrose concentration was higher than 50 g $l^{-1}$ because of high osmotic pressure, which led to browning and even death of explants (Dai et al. 2011).

In plant tissue culture, explant browning is harmful to the cultures and can cause the death of explants. In this study, secondary somatic embryo formation was observed on browned somatic embryos (Fig. 1L). A similar phenomenon has been reported in other species. In *Gossypium hirsutum*, for example, Li et al. (2006) found that somatic embryos were produced from browned calluses. In our study on *Fraxinus mandshurica*, we found that somatic embryos were produced on browned explants (as showed in Fig. 4A, D in Kong et al. 2012). Liu (2009) calculated that about 91.5% of somatic embryos were produced from browned explants. Explant browning of *Eucalyptus globulus* was inhibited but its SE potential was also severely reduced after anti-browning agents were added during the induction period (Pinto et al. 2008). These researches imply

that explant browning might be favorable to SE but the mechanism involved in this process remains unknown.

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