Plant Biotechnology 29, 311–314 (2012)
DOI: 10.5511/plantbiotechnology.12.0330a

Short Communication

Somatic embryogenesis in leaf tissue culture of Soapberry (*Sapindus mukorossi* Gaertn.)

Hyun-Tae Kim[1,2,3], Byeung-Hoon Yang[1,4], Young Goo Park[2,*], Jang R. Liu[3,*]

[1]Department of Forest Genetics, Kyungpook National University, Daegu 702-701, Korea; [2]Department of Forest Biotechnology, Korea Forest Research Institute, Suwon 441-350, Korea; [3]Greenbio Research Center, Korea Research Institute of Bioscience and Biotechnology (KRIBB), Daejeon 305-806, Korea; [4]Forest Environment Conservation Division, Korea Forest Service, Daejeon 302-701, Korea
*E-mail: ygpark@kyungpook.ac.kr jrliu@kribb.re.kr Tel: +82-53-950-5747 Fax: +82-53-950-6708

Received January 5, 2012; accepted March 30, 2012 (Edited by G.-T. Kim)

Abstract Leaf explants formed embryogenic calluses at a frequency of 53.9% when cultured on B5 media supplemented with 0.1 mgl^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.01 mgl^{-1} 6-benzyladenine (BA) for 6 weeks. Upon transfer onto media with 5 mgl^{-1} abscisic acid, embryogenic calluses yielded somatic embryos at 73%. Somatic embryos developed into plantlets on media without plant growth regulators at 90%. Embryogenic calluses proliferated and maintained embryogenic capacity when subcultured on media with 0.1 mgl^{-1} 2,4-D and 0.01 mgl^{-1} BA at 4-week intervals. This culture system is an effective means for clonal propagation and genetic manipulation of soapberry because it ensures taproot development required for tree stability.

Key words: Embryogenic callus, plant regeneration, *Sapindus mukorossi*, somatic embryo.

Soapberry (*Sapindus mukorossi* Gaertn.) is a deciduous tree that has grown in tropical and subtropical zones of China for thousands of years. This tree species bears hard, round, blackish purple fruits. The fruit is used as an expectorant, emetic, and contraceptive, and for treatment of excessive salivation, epilepsy, hypochromic anemia, head lice, migraines, eczema, psoriasis, and freckles (Kirtikar and Basu 1991). Its active ingredients are saponins, for which some chemical identities have been reported (Kuo et al. 2005; Huang et al. 2008).

Soapberry can reproduce through seeds; however, germination is very slow and seedlings have low survival. New plants can also be developed from stem cuttings, yet the cuttings do not develop a taproot necessary for tree stability, thus survival in the field is low (Bhardwaj et al. 1985). Micropropagation of shoot tips excised from seedlings was shown by Philomina and Rao (2000), with the same taproot problem.

Somatic embryogenesis would provide an efficient clonal propagation method that can ensure a taproot system. Recently, somatic embryogenesis of soapberry was reported from leaf explants by Philomina (2010). However, it was not clearly demonstrated that the pattern of development resembles the pattern of zygotic embryogenesis, which is critical in determining whether plant regeneration is achieved via somatic embryogenesis. In this study we established a system for somatic embryogenesis of soapberry from leaf explants, demonstrating a distinct developmental pattern in somatic embryogenesis.

Nut seeds of soapberry (*S. mukorossi* Gaertn.) were obtained from a 3- to 4-m high tree growing at the campus of Kyungpook National University (Daegu, Korea). Mature zygotic embryos were excised from the seeds by cracking, and disinfected by immersion in 70% ethanol for 3 min, followed by 1.05% sodium hypochlorite (NaOCl) with 0.1% Tween-20 for 20–30 min. The embryos were rinsed 3–4 times with distilled water.

Seeds were grown on B5 basal media (Gamborg et al. 1968) containing 3% sucrose solidified with 0.4% Gelrite. The pH was adjusted to 5.8 before autoclaving at 121°C for 15 min. We used 20 ml of media per 87×15 mm plastic Petri dish. Cultures were maintained at 25°C in the light (approximately 15 μmol m^{-2} s^{-1} cool white fluorescent lamps at 16-h photoperiods), for seedling growth and plant regeneration, and, in the dark, for callus induction and proliferation.

Ten disinfested zygotic embryos were placed on each Petri dish of B5 media without plant growth regulators. Leaves were excised from 5- to 14-day-old seedlings and cut into approximately 1×2 cm explants. To induce

Abbreviations: ABA, abscisic acid; BA, 6-benzyladenine; 2,4-D, 2,4-dichlorophenoxyacetic acid; NAA, 1-naphthaleneacetic acid (NAA).
This article can be found at http://www.jspcmb.jp/
Published online May 30, 2012

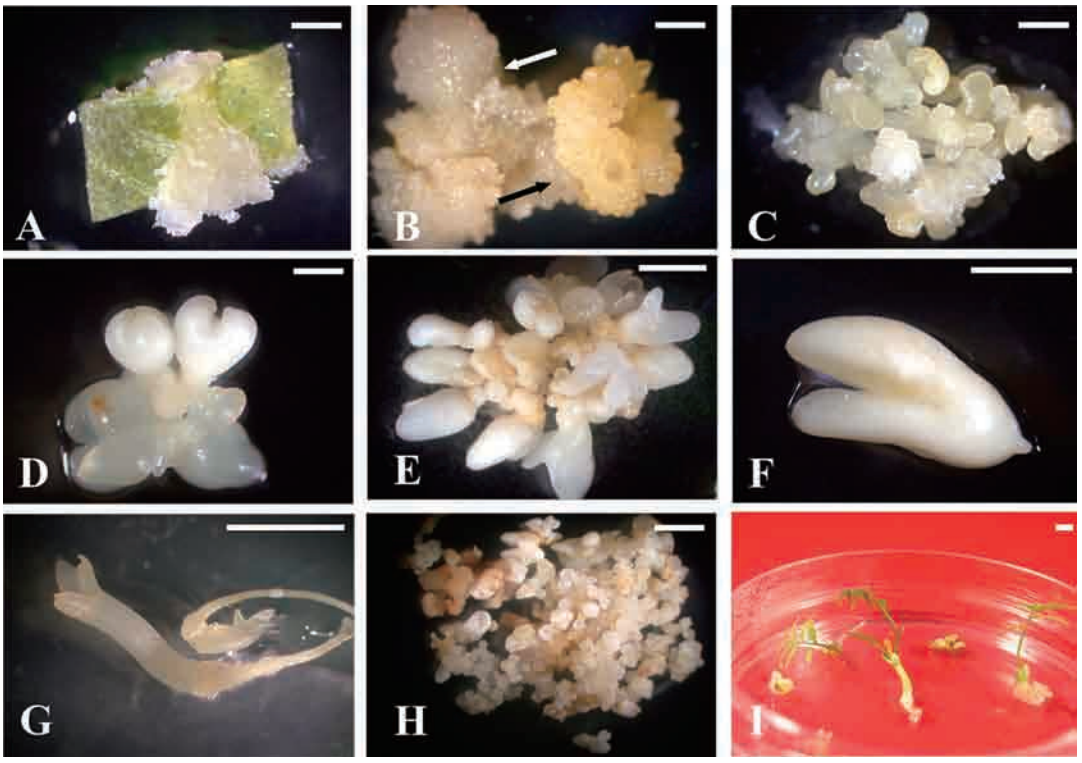

Figure 1. Somatic embryogenesis in leaf tissue culture of *S. mukorossi*. (A) Callus formation on leaf tissue; (B) embryogenic (closed arrow) and nonembryogenic calluses (open arrow); (C) globular shaped embryos; (D, E) heart-shaped embryos; (F) torpedo-shaped embryos; (G) plantlet developed from somatic embryo; (H) proliferation of embryogenic calluses with somatic embryos; (I) plantlets regenerated from somatic embryos. Bar=5 mm.

embryogenic calluses, 10 leaf explants were placed on each Petri dish of B5 medium supplemented with 0.1, 1, 2, or 5 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), with six replicates (Petri dishes) of each. In addition, combinations of 2,4-D and 6-benzyladenine (BA) at a 10:1 concentration were added to media to enhance the frequency of embryogenic callus formation.

To induce somatic embryo maturation, embryogenic calluses with immature somatic embryos were transferred to B5 media supplemented with 0.1, 0.5, 1, 2, or 5 mg l^{-1} abscisic acid (ABA). Mature somatic embryos were transferred to B5 media, without plant growth regulators, to regenerate plantlets. Each concentration treatment of plant growth regulators consisted of 4–7 replicates, with 10 explants each. Data are reported as a mean±standard error (S.E.).

Zygotic embryos germinated into seedlings on media without plant growth regulators. Supplementation of up to 1 mg l^{-1} BA promoted shoot elongation from seedlings (data not shown). Leaf explants excised from seedlings formed calluses on the surfaces when placed on media with 2,4-D (Figure 1A). After 5–6 weeks of culture, two distinct types of calluses were observed: one was yellow and compact, and the other was pale brown and friable (Figure 1B). Globular to heart-shaped somatic embryos were observed on the yellow compact calluses (Figures 1C–E), whereas no organized structures were formed on the pale-brown calluses, even when calluses were cultured for a prolonged period. We, therefore, recognized that the yellow and compact calluses were embryogenic, whereas the pale-brown, compact calluses were not embryogenic.

The frequency of embryogenic callus formation was reduced as 2,4-D concentration was increased (Table 1). Optimum level of 2,4-D for embryogenic callus formation was as low as 0.1 mg l^{-1}, which is considered approximately 10× lower than other species for embryogenic callus formation on leaf explants studied in our laboratory, such as sweet potato (Liu and Cantliffe 1984) and *Hylomecon vernalis* (Kim et al. 2003). The frequency of embryogenic callus formation was enhanced up to approximately 4× at 2,4-D, in combination with BA, as compared to 2,4-D alone (Table 2). The frequency reached a maximum of 53.9% at 0.1 mg l^{-1} 2,4-D and 0.01 mg l^{-1} BA. Leaf explants cultured on media with either 1-naphthaleneacetic acid (NAA) or BA as a sole growth regulator produced only nonembryogenic calluses (data not shown), indicating that 2,4-D may be a prerequisite to embryogenic callus formation in this species, which is contradictory to findings that *Pinellia tripartite* requires BA and NAA, but not 2,4-D (Kim et al. 2003). In addition, in future studies, it is necessary to investigate whether soapberry zygotic embryos are competent for somatic embryogenesis, as

Table 1. Effect of 2,4-D concentration on embryogenic callus and somatic embryo in leaf explants cultures of *S. mukorossi*.

2,4-D (mg l^{-1})	Embryogenic callus formation (%)[a]
0.1	23.4±1.4
1	10.3±2.0
2	6.7±0.5
5	6.6±0.4

[a] Mean±S.E. (n=6).

Table 2. Effect of combination and concentration of 2,4-D and BA on embryogenic callus formation in leaf tissue cultures of *S. mukorossi*.

Growth regulator (mg l^{-1})		Embryogenic callus formation (%)[a]
2,4-D	BA	
0.1	0.01	53.9±4.4
0.5	0.05	43.6±8.9
1	0.1	30.8±13.3
2	0.2	12.8±11.8
5	0.5	7.7±7.7

[a] Mean±S.E. (n=4).

Table 3. Effect of ABA concentration on maturation of somatic embryos in *S. mukorossi*.

ABA (mg l^{-1})	Mature somatic embryo formation (%)[a]
0.1	40.9±6.0
0.5	49.7±6.9
0	31.9±5.0
1	56.3±6.8
2	57.5±4.1
5	73.3±8.1

[a] Mean±S.E. (n=7).

exemplified in *Nymphoides coreana* (Oh et al. 2010) and radiata pine (Montalbán et al. 2011).

ABA promoted maturation of somatic embryos as its concentration was increased (Table 3; Figures 1F, G). The frequency of maturation reached 73% at 5 mg l^{-1} ABA. Embryogenic calluses proliferated, producing somatic embryos on the surfaces when sub-cultured on media with 0.1 mg l^{-1} 2,4-D and 0.01 mg l^{-1} BA at 4-week intervals (Figure 1H). ABA is useful in promoting the transition of somatic embryos from the proliferation to the maturation phase and in enhancing embryo quality by preventing precocious germination (Rai et al. 2011). Mature embryos developed into plantlets, when transferred onto media without plant growth regulators, at a frequency of 90% (Figure 1I).

Soapberry has been clonally propagated *in vitro* by seedling-derived shoot-tip culture (Philomina and Rao 2000). Clonal plants produced with this method, however, do not produce an adequate taproot system required for both enhanced drought tolerance at the seedling stage (Poorter and Markesteijn 2008) and physical stability at maturation. Such a problem can be overcome by plant regeneration via somatic embryogenesis. Plants developed from somatic embryos in this study could have the same taproot system as plants germinated from seeds.

Although Philomina (2010) reported somatic embryogenesis from leaf explants of soapberry, it was not clearly demonstrated that the pattern of development follows the same as zygotic embryos, which is critical in determining whether plant regeneration is achieved via somatic embryogenesis. In addition, no torpedo-shaped embryo stage was described in which morphological bipolarity is established. An embryonic bipolar structure is prerequisite to the development of a taproot. Furthermore, their cotyledonary somatic embryos were vulnerable to revert to callusing, suggesting that the canalization of somatic embryos into a regular plant development is not fully established.

In this study we established a system for somatic embryogenesis in leaf explants cultures of soapberry, demonstrating a typical developmental pattern of somatic embryogenesis with a high degree of developmental stability. It was also possible to subculture embryogenic calluses with a maintained embryogenic capacity. This culture system can provide a means for clonal propagation and genetic manipulation of soapberry while ensuring the development of a taproot.

Acknowledgements

This work was supported by a grant to J.R.L. from the Technology Development Program for Agriculture and Forestry funded by the Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries, a grant to J.R.L. from the Marine Extreme Genome Research Center funded by the Korean Ministry of Marine Affairs and Fisheries, a grant to J.R.L. from the Advanced Biomass R&D Center of Korea Grant funded by the Ministry of Education, Science and Technology (ABC-2010-0029723), and a grant to J.R.L. from Korea Research Institute of Bioscience and Biotechnology for the Development of Cell Factory for Biomass and Bio-energy.

References

Bhardwaj SD, Verma AK, Bawa R (1985) Package of practices for forestry crops (trees, shrups, grasses, bamboos and medicinal & aromatic plants). Director, Directorate of Extension Education, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan (HP)

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. *Exp Cell Res* 50: 151–158

Huang HC, Wu MD, Tsai WJ, Liao SC, Liaw CC, Hsu LC, Wu YC, Kuo YH (2008) Triterpenoid saponins from the fruits and galls of *Sapindus mukorossi*. *Phytochemistry* 69: 1609–1616

Kim SW, Oh SC, Kim TJ, Liu JR (2003) High frequency somatic embryogenesis and plant regeneration in petiole and leaf explant cultures and petiole-derived embryogenic cell suspension cultures of *Hylomecon vernalis* Max. *Plant Cell Tissue Organ Cult*

74: 163–167

Kirtikar KR, Basu BD (1991) *Indian Medicinal Plants*, B.L.M. Publication, Allahabad, pp 897–898

Kuo YH, Huang HC, Yang Kuo LM, Hsu YW, Lee KH, Chang FR, Wu YC (2005) New dammarane-type saponins from the galls of *Sapindus mukorossi*. *J Agric Food Chem* 53: 4722–4727

Liu JR, Cantliffe DJ (1984) Somatic embryogenesis and plant regeneration in tissue cultures of sweet potato (*Ipomoea batatas* Poir.). *Plant Cell Rep* 3: 112–115

Montalbán IA, De Diego N, Igartua EA, Setién A, Moncaleán P (2011) A combined pathway of somatic embryogenesis and organogenesis to regenerate radiata pine plants. *Plant Biotechnol Rep* 5: 177–186

Oh MJ, Na HR, Choi HK, Liu JR, Kim SW (2010) High frequency plant regeneration system for *Nymphoides coreana* via somatic embryogenesis from zygotic embryo-derived embryogenic cell suspension cultures. *Plant Biotechnol Rep* 4: 125–128

Philomina NS (2010) Somatic embryogenesis from leaf explants of soapnut (*Sapindus mukorossi* Gaertn.). *Indian J Biotechnol* 9: 336–337

Philomina NS, Rao JVS (2000) Micropropagation of *Sapindus mukorossi* Gaertn. *Indian J Exp Biol* 38: 621–624

Poorter L, Markesteijn L (2008) Seedling traits determine drought tolerance of tropical tree species. *Biotropica* 40: 321–331

Rai MK, Shekhawat NS, Harish, Gupta AK, Phulwaria M, Ram K, Jaiswal U (2011) The role of abscisic acid in plant tissue culture: a review of recent progress. *Plant Cell Tissue Organ Cult* 106: 179–190