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

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Abstract Leaf explants formed embryogenic calluses at a frequency of 53.9% when cultured on B5 media supplemented with 0.1 mg l\(^{-1}\) 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.01 mg l\(^{-1}\) 6-benzyladenine (BA) for 6 weeks. Upon transfer onto media with 5 mg l\(^{-1}\) 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 mg l\(^{-1}\) 2,4-D and 0.01 mg l\(^{-1}\) 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 x 15 mm plastic Petri dish. Cultures were maintained at 25°C in the light (approximately 15 \(\mu\)mol 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...
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-\(\text{d}\)), with six replicates (Petri dishes) of each. In addition, combinations of 2,4-\(\text{d}\) 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-\(\text{d}\) (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-\(\text{d}\) concentration was increased (Table 1). Optimum level of 2,4-\(\text{d}\) for embryogenic callus formation was as low as 0.1 mg l\(^{-1}\), which is considered approximately 10\(\times\) 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\(\times\) at 2,4-\(\text{d}\), in combination with BA, as compared to 2,4-\(\text{d}\) alone (Table 2). The frequency reached a maximum of 53.9% at 0.1 mg l\(^{-1}\) 2,4-\(\text{d}\) 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-\(\text{d}\) 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-\(\text{d}\) (Kim et al. 2003). In addition, in future studies, it is necessary to investigate whether soapberry zygotic embryos are competent for somatic embryogenesis, as

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.
ABA promotes 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 stage to the maturation phase and in enhancing embryo capability. This culture system can provide a means for clonal propagation and genetic manipulation of soapberry while ensuring the development of a taproot.

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