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Somatic Embryogenesis and Plantlet Formation from a Rare and Endangered Tree Species, *Oplopanax elatus*

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We tested the possibility of plantlet formation via somatic embryogenesis with leaf segments and mature zygotic embryos from a rare and endangered tree species, *Oplopanax elatus*. To induce calli, explants were cultured under darkness in a solid MS medium containing 3% sucrose, 1g L^{-1} glutamine, and 0.3% gelrite. Treatment supplements included 2,4-D alone or in combination with thidiazuron. Generally, callus induction and growth were good from leaf explants, whereas embryogenic calli could be induced only from zygotic embryos. These embryogenic calli were white or pale yellow and very friable. ABA and activated charcoal appeared to be important factors when inducing somatic embryos, with optimum levels being 0.1 mg L^{-1} and 0.02%, respectively. Many somatic embryos showed abnormalities during their development on the germination medium, but 35% could be converted if placed on a medium containing gibberellic acid (GA_3). The germinating embryos sometimes formed secondary embryos at the lower portion of the hypocotyls. Normal or converted plantlets were acclimatized in an artificial soil mixture; their survival was about 60% after two months. This culturing system provides a feasible approach for regenerating plants, via somatic embryogenesis, from mature zygotic embryos.

Keywords: micropropagation, *Oplopanax elatus*, plantlet formation, somatic embryogenesis, zygotic embryos

A member of the Araliaceae, the small genus of *Oplopanax* Miq. includes three species: *Oplopanax horridus*, which grows along the Pacific Coast of the United States and Canada; *Oploxpanax japonicus*, from Japan; and *Oplopanax elatus*, a tree species from the Russian Far East and Korea (Fu and Jin, 1992; Zhuravlev and Kolyada, 1996). The tree is actually a deciduous shrub 1.0 to 1.8 m tall, with thick, spiny stems that arise from a rhizomatous base and large, palmately lobed leaves that form a canopy-like layer. *O. elatus* is a valuable medicinal plant, and was one of the first among Far-Eastern plants to be recommended for research as a ginseng-type preparation source. Because of its highly medicinal properties against such maladies as asthenia, depressive states, and hypertension, demand for this rare and endangered species has increased and mass collection is on-going (Zhuravlev and Kolyada, 1996). Its distribution in Korea is quite limited, occurring only on several high mountains and within small, restricted areas (Lee et al., 2002). Because of its pharmaceutical interest, this species has historically been intensively over-exploited in Korea.

Conventional reproduction by seeds is ineffective and fruit set is poor in its natural habitat. The dormant embryos require at least 18 months of stratification to germinate. Under natural conditions, germination success is only about 5%; when treated with gibberellin, that rate may rise to 30%. In some cases, reproduction is possible via divisions, layering, or cutting, although those methods are not efficient or practical (Fu and Jin, 1992; Zhuravlev and Kolyada, 1996). Moreover, the distribution of their weak, less viable embryos is restricted to high elevations (Lee et al., 2002). Therefore, under *ex vitro* conditions, root cuttings have been the only possible regeneration system, but their seasonal limitations make this method inefficient.

In vitro culture is a desirable, alternative approach for replicating genetically identical plantlets from rare and endangered species (Kowalski and von Staden, 2001; Negash, 2002; Rai, 2002; Gomes et al., 2003; Martin, 2003a, b; Hussein et al., 2006). Although callus induction and somatic embryogenesis have been widely researched in the Araliaceae genera, plant regeneration via normal somatic embryo induction apparently had not been achieved with *O. elatus* (Moon and Youn, 1999; Moon et al., 2005). For this species, callus induction and somatic embryogenesis have been reported only by Cho et al. (1991). Therefore, the objective of this current study was to devise an alternative regeneration system via somatic embryogenesis for the propagation of *O. elatus*.

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MATERIALS AND METHODS

Explants and Surface Disinfection

Expanded leaves of *O. elatus* (15 cm long and 10 cm wide) were collected from greenhouse-grown stock plants that had been raised from rooted propagules. The greenhouse was maintained at 20 to 30°C, under 30% shading. The leaf samples were divided into four pieces (about 5 × 5 cm), washed with tap water and a few drops of Tween 20, then disinfested with 70% ethanol for 3 min and with 2% (w/v) NaOCl (Yakuri Pure, Japan) for 10 min. These explants were rinsed three times with sterile deionized water and dipped for 30 min. After the disinfection process, the pieces were cut into smaller segments (about 5 × 5 mm) and placed, abaxial side down, on a callus induction medium. Mature seeds were collected from Mt. Seolak, Korea, during August of 2003, and transported in an icebox to the laboratory. They were washed in tap water and a few drops of Tween 20, then disinfested with 70% ethanol for 3 min, 2% NaOCl for 30 min, and 0.2% (w/v) $HgCl_2$ (Aldrich, USA) for 20 min before being rinsed four times with sterile distilled water. Only healthy seeds, which manifested normal endosperms under the microscope, were used for these experiments. The seeds were carefully de-husked with a scalpel, and the zygotic embryos were inoculated on a callus induction medium.

Table 1. Effect of basal medium and PGRs on callus and embryogenic tissue from leaves and mature zygotic embryos (seeds) of *O. elatus*.

Medium (mg L^{-1})	% of callus induction[1]		% of embryogenic tissue induction[2]	
	Leaf	seed	leaf	seed
MS+2,4-D 1.0	100	71.0	0	1.2
MS+2,4-D 2.0	100	72.0	0	0.8
MS+2,4-D 1.0, TDZ 0.01	100	36.0	0	0
MS+2,4-D 2.0, TDZ 0.01	100	36.0	0	0

[1]% of callus induction = percentage of leaf and zygotic embryos that formed non-embryogenic calli.
[2]% of embryogenic tissue induction = percentage of leaf and zygotic embryos that formed friable, white embryogenic calli.

Preparation of Callus Induction Medium

Using a slightly modified version of our previous protocol (Moon et al., 2005), we prepared a callus induction medium consisting of an MS medium (Murashige and Skoog, 1962) that contained 1g L^{-1} glutamine, 3% sucrose, and 0.3% gelrite (Aldrich, USA), and which was supplemented with 1.0 or 2.0 mg L^{-1} of 2,4-D (2,4-dichlorophenoxy acetic acid), either alone or in combination with 0.01 mg L^{-1} of TDZ (thidiazuron, *N*-phenyl-*N*-1,2,3-thiadiazol-5-ylurea; Aldrich) (Table 1). Glutamine was added after filtering. The medium was adjusted to pH 5.8 with 0.1N NaOH or HCl before the addition of gelrite. After being autoclaved at 1.05 kg cm^{-2} and 121°C for 20 min, 25 mL of medium was poured into each Petri dish. The distribution of plant materials (per dish) was seven leaf segments (ten replications) or five naked seeds (five replications). Following this inoculation, the cultures were maintained for four weeks at 24 ± 2°C under darkness.

Induction and Proliferation of Embryogenic Calli

After four weeks of initial culturing, explants that had formed calli were transferred to a half-strength MS medium (1/2 MS) containing 2% sucrose and 0.3% gelrite, but without any plant growth regulators, to induce embryogenic calli. Rapid growth was achieved in the medium in the first four weeks, so that by week 8, we observed the development of white, very friable embryogenic calli from the zygotic embryos. These calli were then selected for proliferation on an MS medium (1.0 mg L^{-1} 2,4-D, 1 g L^{-1} glutamine, 5% sucrose, and 0.5% gelrite) under darkness, with sub-culturing at three-week intervals.

Induction of Somatic Embryos

Somatic embryos were induced from our embryogenic calli on 1/2 MS [2% sucrose, 0.02% activated charcoal (Aldrich), and 0.3% gelrite] supplemented with 0.00, 0.05, 0.10, 0.20, or 0.50 mg L^{-1} of (±)-*cis, trans*-ABA (Sigma, USA), which was added after filtering. About 0.5 g of embryogenic calli was inoculated onto each dish, and the torpedo-stage and early cotyledonary-stage embryos were counted after four weeks (Table 2). These cultures were maintained at 24 ± 2°C, with a 16-h photoperiod provided by cool-white fluorescent lamps (40 μmol m^{-2} s^{-1}).

Germination of Somatic Embryos and Plantlet Conversion

Somatic embryos in the early cotyledonary stages were selected and sub-cultured on an MS medium containing various level of GA_3 (Sigma) for germination (Table 3). The gibberellin was added after filtering. About 20 somatic embryos (in six replicates) were cultured on each 1 × 10-cm Petri dish, which contained 25 mL of an MS medium with 3% sucrose and 0.3% gelrite. To foster their growth after conversion, more than 500 plantlets were then transferred to larger vials (10 × 5 cm) containing 30 mL of 1/2 MS but without any PGRs.

Soil Transfer and Acclimatization

Two hundred normally converted young plantlets (about 7 to 10 cm long) were selected from the culture dishes and the agar was carefully removed from the roots with tap water. These plantlets were then transferred to an artificial soil mixture (1:2 PKS2:perlite, by volume) and cultured in a high-humidity greenhouse to determine their survival after two months.

RESULTS

Callus Induction

Our tests proved that surface-disinfection of the seeds is a critical factor in determining their *in vitro* survival. More than 50% were contaminated and had to be discarded within the first week of culturing (data not shown). Therefore, to continue with such an approach, it will be necessary to develop a more efficient disinfection technique for rescuing seeds or other explant sources. As was also found from our previous research (Moon and Youn, 1999), leaf explants here proved to be a suitable alternative, and contamination from microorganisms was not a problem when those tissues were taken from greenhouse-grown stock plants.

After one week of culturing, calli began forming at the cut surfaces of the leaf segments, especially from the leaf veins (Fig. 1A). Most were compact and light green to yellow. In a separate experiment, calli also appeared from the swollen tissue of zygotic seed embryos after three weeks of culturing (Fig. 1B). These, however, were soft and white or pale brown. Treatment with 2,4-D alone was better for inducing calli from zygotic embryos than when used in combination with TDZ (Table 1). However, callus growth was faster in response to that double source of cytokinin, and some seeds germinated directly and grew into plantlets without any intermediate callus stage.

Induction of Embryogenic Calli and Somatic Embryos

After one month of culturing, calli induced from the leaf segments had proliferated but then turned brown on the 1/2 MS medium; these were non-embryogenic. When transferred to fresh 1/2 MS, they still remained unviable for three months, exuding phenolic compounds, and gradually dying. Although we added 0.02% activated charcoal to remove those compounds, the state of these calli was not recoverable. In contrast, calli induced from mature seeds were capable of producing somatic embryos through the formation of typical embryogenic cells. Those calli were soft and white, with fragile cells (Fig. 1C).

After the embryogenic calli were separated out from the non-productive calli, they were maintained for more than two years on an MS medium supplemented with 1.0 mg L^{-1} 2,4-D, 1.0 g L^{-1} glutamine, 5% sucrose, and 0.5% gelrite, with sub-culturing at three-week intervals. Those, however, that turned brown over

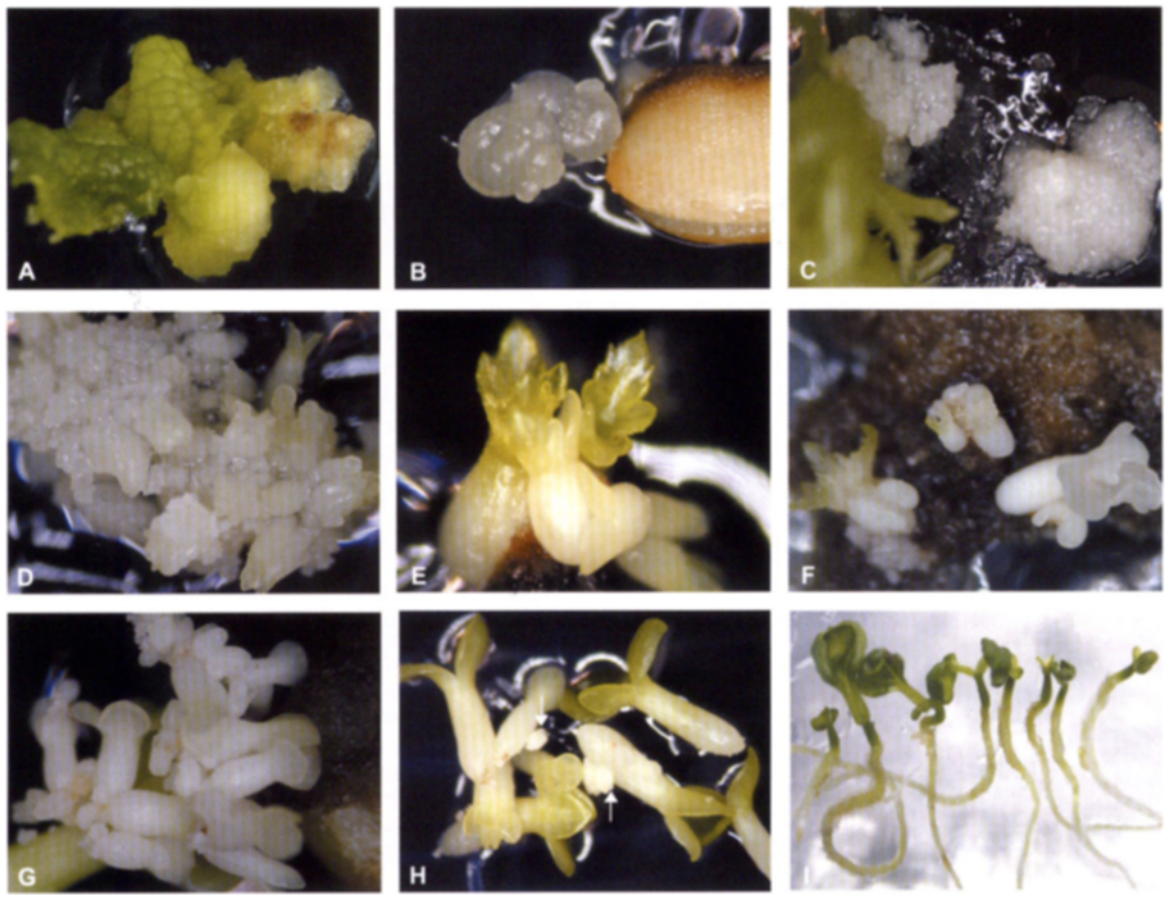

Figure 1. Somatic embryogenesis and plant regeneration of *O. elatus*. **A**, Callus induced from leaf explant; **B**, Embryogenic tissue induced from zygotic embryo after 8 weeks in culture; **C**, Representative embryogenic calli that are friable, milky, and white; **D**, Different stages achieved by somatic embryos developed from embryogenic calli; **E**, Mature somatic embryos with well-developed cotyledons and elongated hypocotyls; **F**, Directly induced somatic embryos from surface of browning callus; **G**, Directly induced somatic embryos from lower portion of hypocotyl in mature somatic embryo; **H**, Normally matured somatic embryos showing cotyledon development. Arrow indicates secondary somatic embryos; **I**, Germinating somatic embryos with elongated hypocotyl and root.

this period were discarded during the sub-culturing process.

When ABA was excluded from the basal medium, the induction rate was very low, with most of the ensuing somatic embryos being abnormal (Table 2). However, in the presence of ABA, induction was normal, averaging more than 100 embryos per dish, regardless of the concentration applied. These induced somatic embryos grew to different developmental stages, some achieving the early cotyledon phase (Fig. 1D, E). An ABA concentration of 0.1 mg L^{-1} appeared optimal for attaining further maturation; most somatic embryos reached the torpedo stage but additional growth was suppressed in the presence of 0.5 mg L^{-1} ABA (Table 2). Secondary somatic embryos developed prematurely when formed from the browning embryonic tissue (Fig. 1F).

Table 2. Effect of ABA on somatic embryo induction from embryogenic calli.

Medium[1] (mg L^{-1})	No. of torpedo embryos/plate	No. of early cotyledonary embryos/plate
1/2MS control	20<	2.0 ± 0.5[2]
1/2MS+ABA 0.05	100<	3.3 ± 0.9
+ABA 0.1	100<	6.8 ± 2.4
+ABA 0.2	100<	1.8 ± 1.2
+ABA 0.5	100<	0.3 ± 0.02

[1]All media were supplemented with 0.02% activated charcoal.
[2]Values shown are means ± standards deviations for data obtained from 5 replicate experiments in each treatment.

Germination and Regeneration of Somatic Embryos

Embryos at the early-cotyledonary stage were transferred to MS media supplemented with various level of GA_3 (Fig. 1H).

Table 3. Effect of GA_3 on somatic embryo germination and plantlet conversion.

Medium (mg L^{-1})	No. of embryos cultured[1]	No. of embryos germinated (%)[2]	No. of embryos converted (%)[3]
MS control	130	106 (81.5)	13 (10.0)
MS +GA_3 1.0	130	126 (96.9)	32 (24.6)
+GA_3 3.0	123	116 (94.3)	33 (26.8)
+GA_3 5.0	133	131 (98.3)	47 (35.3)
+GA_3 10.0	122	109 (89.3)	18 (14.3)

[1]Early cotyledonary-stage embryos were cultured.
(%)[2], Percentage of embryos with elongated hypocotyls and root growth, but without normally expanded leaves.
(%)[3], Percentage of plantlets with normally expanded leaves, elongated hypocotyls, and root growth.

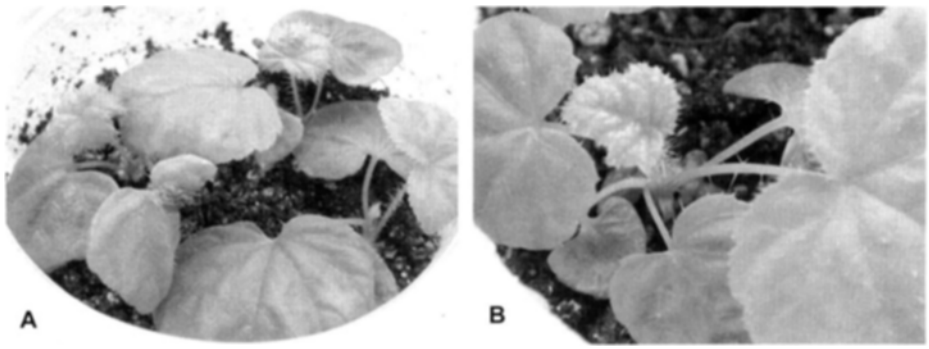

Figure 2. Acclimatized plantlets: **A**, one-month-old; **B**, two-month-old.

These embryos easily germinated in all tested media except the control, and showed elongated hypocotyls and rooting within one week (Fig. 1I). Germination rates were 89 to 96% from the treated media (Table 3). Nevertheless, many of those exhibited abnormalities, including multi-cotyledon embryos, fused cotyledons, and/or embryos with roots only. Among the somatic embryos that failed to germinate, secondary somatic embryos often formed on the lower portions of the hypocotyls during the germination process. These appeared to be produced directly from the hypocotyl surface, without intermediate callusing (Fig. 1G). In contrast to the high germination frequencies, the rates of plantlet conversion were poor from 14.3 to 35.5%. Most of the abnormally germinating embryos could not be converted to plantlets. From preliminary anatomical observations, we noted that although the germinated embryos possessed apical meristems, they were unable to regenerate plantlets because their cotyledons were fused (data not shown). Growth of the converted plantlets and their leaf expansion were stimulated by sub-culturing those tissues in larger vessels containing 1/2 MS, 2% sucrose, and 0.02% activated charcoal.

These converted plantlets were eventually transferred to an artificial soil mixture and acclimatized in the greenhouse. Growth was normal, without any morphological deficiencies (Fig. 2). Among our 200 transferred plantlets, 119 survived (about 60% survival rate) after 2 months.

DISCUSSION

As an auxin source, 2,4-D is generally used to induce somatic embryo development under *in vitro* conditions (Feher et al., 2003; Jimenez, 2005; Moon et al., 2005). A combination of auxin and cytokinin or cytokinin alone is applied to the tissue culture medium, depending upon the species of interest (Bonneau et al., 1994; Das et al., 1997; Merkle et al., 1997; Nanda and Rout, 2003; Vookova et al., 2003). Here, treatment with 2,4-D alone was better than in combination with cytokinin for our *in vitro* culturing of *O. elatus*. Although the frequency of callus induction did not differ between single and double treatments of auxin and cytokinin, embryogenic calli were formed only with the single treatment of 2,4-D (Table 1). Such results have also been reported in many other somatic embryogenesis systems (Vendrame et al., 2001; Choi and Jeong, 2002; Kim et al., 2003; Martin, 2003b; Moon et al., 2005).

The juvenility of the plant tissue and the choice of explant type are usually the most important factors in determining the regenerability of plantlets from woody species (Merkle et al., 1997, 1998; Chalupa, 2000; Bonga, 2004). In our study, non-embryogenic calli were induced from leaf segments, whereas embryogenic calli, capable of plant regeneration, were induced only from mature zygotic embryos. This suggests that selecting the proper explant source is critical to the successful induction of embryogenic calli in *O. elatus*. Merkle and Battle (2000) have reported that, for somatic embryogenesis of mature sweetgum, staminate inflorescences are up to five times more likely to produce embryogenic calli than are female inflorescences. Furthermore, although Cho et al. (1991) have been able to induce embryogenic calli from the inflorescence tissue of *O. elatus,* they have been unable to obtain plantlets from somatic embryos.

In our study, some zygotic embryos germinated directly on the callus induction media, without callusing, and then regenerated plantlets when sub-cultured on a hormone-free MS medium. This might have resulted because the mechanical dormancy of the seeds was broken when their coats were first de-husked. Seeds of this species generally have a long period of embryo dormancy that must be interrupted by stratification for at least two years before germination can occur. Therefore, if we could use *in vitro* manipulation of these seeds to achieve higher germination rates, that method would be desirable for producing future plantlets *in vitro*. However, it is difficult to collect viable seeds from their normally restrictive mountain habitat at high elevations (Zhuravlev and Kolyada, 1996).

ABA is an important growth regulator for embryo maturation, and is an effective additive in media that support the growth of globular and early, heart-shaped somatic embryos (Capuana and Debergh, 1997). This PGR prevents precocious embryo germination and inhibits recurrent embryogenesis, and can promote the accumulation of reserve substances such as protein and lipids in *Picea* (Hackman and von Arnold, 1988). Here, ABA treatment was effective for inducing somatic embryos, although numerous abnormalities also occurred. Thus, it will be necessary to use embryo synchronization as well as ABA treatment for enhancing normal embryo germination. Dai et al. (2004) and Tonon et al. (2001) have suggested that such synchronization, via cell fractions and thieving, may help increase embryo germination rates in several woody species. Therefore, further experiments are needed to establish a micropropagation system for species using somatic embryogenesis.

The maturation, germination, and conversion of somatic embryos into plants are difficult tasks during somatic embryogenesis (Sutton and Polonenko, 1999). For woody species especially, the efficiency of conversion is relatively low, hampering the application of somatic embryogenesis systems for

purposes of commercialization (Merkle et al., 2003). Our highest conversion rate for somatic embryos was 35% (Table 3). Therefore, we must, in future, increase this efficiency so that we can better produce normal plantlets.

Two kinds of somatic embryogenesis pathways exist in plants: IEDC (induced embryogenic determined cells) and PEDC (pre-embryogenic determined cells) (Sharp et al., 1980). Both are capable of inducing somatic embryos in *O. elatus*. In our study, primary somatic embryos were induced by calli differentiation from zygotic embryos, while secondary somatic embryos were produced directly from the hypocotyls of those primary embryos, without intermediate callusing during sequential sub-culturing (Fig. 1G, H). This latter case might make it possible to form cyclic somatic embryos with higher efficiency of regeneration, and could be used as a mass-propagation tool for various plants, including woody species (Raemakers et al., 1999; Vendrame et al., 2001). The secondary embryos in our experiments were easily separated from the mother tissues, and germinated rapidly after cotyledon expansion when cultured on an MS medium supplemented with 1.0 mg L^{-1} GA_3. However, most of those secondary somatic embryos formed sporadically and needed to be selected each time for germination, making that process laborious and time-consuming.

Normally converted plantlets (about 7 to 10 cm long) were transferred to an artificial soil mixture and acclimatized in the greenhouse, with a two-month survival rate of about 60% (Fig. 2). Much of their mortality may have been due to high-temperature stress in the summer season because, in their natural, mountainous habitat, plants grow at high elevations with cool conditions and high humidity. Therefore, future research will need to identify the necessary steps for improving survival rates when *in vitro*-raised plantlets are transferred to soil.

For many rare and endangered plant species, *in vitro* regeneration has been accomplished through organogenesis, using axillary bud and callus cultures (Negash, 2002; Rai, 2002; Gomes et al., 2003; Martin, 2003a). Somatic embryogenesis of such species is not as common (Augustine and D' Souza, 1997; Beena and Martin, 2003). However, the latter method is preferred over adventitious regeneration because it can be efficient in reducing time and cost factors (Moon and Youn, 1999; Merkle et al., 1997, 2003; Bodhipadma and Leung, 2002; Moon et al., 2005). Although our conversion rates were not optimal, we were able to demonstrate here that micropropagation of *O. elatus* is feasible via somatic embryogenesis.

In conclusion, we have now developed a regeneration system that relies on somatic embryogenesis from mature zygotic embryos in *O. elatus*. However, the rates for embryogenic callus induction were very low and the frequency of conversion was unsatisfactory. Therefore, further, detailed study is needed in this area. One other difficulty arose because viable seed of this species is not easily collected from its natural habitat. This means that embryogenesis must rely on other somatic tissues, e.g., leaves, stems, and roots. Although we were unable to induce embryogenic callus formation from leaf segments, we could obtain somatic embryos from leaf and petiole segments when using *in vitro*-grown plants. Further experiments will be necessary to establish an intact protocol for somatic embryogenesis with this species. Nevertheless, our results are the first reported for *in vitro* plant regeneration via somatic embryogenesis in *O. elatus*. We expect that this method will be feasible for efficient micropropagation as well as germplasm conservation of such a valuable medicinal woody species.

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LITERATURE CITED

Augustine AC, D' Souza L (1997) Somatic embryogenesis in *Gnetum ula* Brongn. (*Gnetum edule*) (Willd) Blume. Plant Cell Rep 16: 354-357

Beena MR, Martin KP (2003) *In vitro* propagation of the rare medicinal plant *Ceropegia candelabrum* L. through somatic embryogenesis. In Vitro Cell Dev Biol Plant 39: 510-513

Bodhipadma K, Leung DWM (2002) Factors important for somatic embryogenesis in zygotic embryo explants of *Capsicum annuum* L. J Plant Biol 45: 49-55

Bonga JM (2004) The effect of various culture media on the formation of embryo-like structures in cultures derived from explants taken from mature *Larix deciduas*. Plant Cell Tiss Org Cult 77: 43-48

Bonneau L, Beranger-Novat N, Monin J (1994) Somatic embryogenesis and plant regeneration in a woody species: The European spindle tree (*Euonymus europaeus* L.). Plant Cell Rep 13: 135-138

Capuana M, Debergh PC (1997) Improvement of the maturation and germination of horse chestnut somatic embryos. Plant Cell Tiss Org Cult 48: 23-29

Chalupa V (2000) *In vitro* propagation of mature trees of pedunculate oak (*Quercus robur* L.). J Forest Sci 46: 537-542

Cho HJ, Yang WY, Yun DW, Kim YH, Soh WY, Chung TY (1991) Somatic embryogenesis from inflorescence derived callus of *Oplopanax elatus* Nakai. Res Rep RDA B Kor 33: 1-6

Choi YE, Jeong JH (2002) Dormancy induction of somatic embryos of Siberian ginseng by high sucrose concentrations enhances the conservation of hydrated artificial seeds and dehydrated resistance. Plant Cell Rep 20: 1112-1116

Dai J, Vendrame WA, Merkle SA (2004) Enhancing the productivity of hybrid yellow-poplar and hybrid sweetgum embryogenic cultures. In Vitro Cell Dev Biol Plant 40: 376-383

Das P, Samantaray S, Roberts AV, Rout GR (1997) *In vitro* somatic embryogenesis of *Dalbergia sissoo* Roxb.- a multipurpose timber-yielding tree. Plant Cell Rep 16: 578-582

Feher A, Taras P, Pasternak P, Dudits D (2003) Transition of somatic plant cells to an embryogenic state. Plant Cell Tiss Org Cult 74: 201-228

Fu LK, Jin J (1992) China Plant Red Data Book: Rare and Endangered Plants. Science Press, Beijing, pp 741

Gomes GAC, Paiva R, Paiva PDDO, Santiago EJAD (2003) Plant regeneration from callus cultures of *Maclura tinctoria*, an endangered woody species. In Vitro Cell Dev Biol Plant 39: 293-295

Hackman I, von Arnolds (1988) Somatic embryogenesis and plant regeneration from suspension cultures of *Picea glauca* (white spruce). Physiol Plant 72: 579-587

Hussein S, Ibrahim R, Kiong ALP (2006) Potential biochemical markers for somatic embryos of *Eurycoma logifolia* Jack. J Plant Biol 49: 97-101

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

Kim SW, Oh SC, Liu JR (2003) Control of direct and indirect

somatic embryogenesis by exogenous growth regulators in immature zygotic embryo cultures of rose. Plant Cell Tiss Org Cult 74: 61-66

Kowalski B, von Staden J (2001) *In vitro* culture of two threatened South African medicinal trees - *Ocotea bullata* and *Warburgia salutaris*. Plant Growth Regul 34: 223-228

Lee SW, Kim YM, Kim WW, Chung JM (2002) Genetic variation of I-SSR markers in the natural populations of a rare and endangered tree species, *Oplopanax elatus* in Korea. J Kor For Soc 91: 565-573

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

Martin KP (2003b) Plant regeneration through somatic embryogenesis on *Holostemma adakodien*, a rare medicinal plant. Plant Cell Tiss Org Cult 72: 79-82

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

Merkle SA, Battle PJ (2000) Enhancement of embryogenic culture initiation from tissues of mature sweetgum trees. Plant Cell Rep 19: 268-273

Merkle SA, Battle PJ, Ware GO (2003) Factors influencing production of inflorescence-derived somatic seedlings of sweetgum. Plant Cell Tiss Org Cult 73: 95-99

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

Moon HK, Kim YW, Lee JS, Choi YE (2005) Micropropagation of *Kalopanax pictus* tree via somatic embryogenesis. In Vitro Cell Dev Biol Plant 41: 303-306

Moon HK, Youn Y (1999) Somatic embryogenesis from winter buds of 10-year-old *Aralia elata*, *In* SM Jain, PK Gupta, RJ Newton, eds, Somatic Embryogenesis in Woody Plants. Vol 5, Kluwer, Dordrecht, pp 129-134

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue. Physiol Plant 15: 473-479

Nanda RM, Rout GR (2003) *In vitro* somatic embryogenesis and plant regeneration in *Acacia arabica*. Plant Cell Tiss Org Cult 73: 131-135

Negash L (2002) Successful vegetative propagation techniques for the threatened African pencil cedar (*Juniperus procera* Hoechst. Ex Endl.). For Ecol Manag 161: 53-64

Raemakers K, Jacobsen E, Visser R (1999) Proliferative somatic embryogenesis in woody species, *In* SM Jain, PK Gupta, RJ Newton, eds, Somatic Embryogenesis in Woody Plants. Vol 4, Kluwer, Dordrecht, pp 29-59

Rai VR (2002) Rapid clonal propagation of *Nothapodytes foetida* (Wight) Sleumer–a threatened medicinal tree. In Vitro Cell Dev Biol Plant 38: 347-351

Sharp WR, Sondahl MR, Caldas LS, Maraffa SB (1980) The physiology of *in vitro* asexual embryogenesis. Hortic Rev 2: 268-310

Sutton BSC, Polonenko DR (1999) Commercialization of plant somatic embryogenesis, *In* SM Jain, PK Gupta, RJ Newton, eds, Somatic Embryogenesis in Woody Plants. Vol 4, Kluwer, Dordrecht, pp 263-291

Tonon G, Berardi G, Rossi C, Bagnaresi U (2001) Synchronized somatic embryo development in embryogenic suspensions of *Fraxinus angustifolia*. In Vitro Cell Dev Biol Plant 37: 462-465

Vendrame WA, Holliday CP, Merkle SA (2001) Clonal propagation of hybrid sweetgum (*Liquidambar styraciflua* x *L. formosana*) by somatic embryogenesis. Plant Cell Rep 20: 691-695

Vookova B, Matusova R, Kormutak A (2003) Secondary somatic embryogenesis in *Abies numidica*. Biol Plant 46: 513-517

Zhuravlev Yu N, Kolyada AS (1996) Araliaceae: Ginseng and Other Aralia of the Russian Far East. Dalnauka Press, Vladivostok