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Somatic Embryogenesis, Plant Regeneration, and Field Establishment from Tissue Culture of Winter Buds of 10-year-old *Aralia elata*[1]

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10年生 두릅나무의 冬芽를 利用한 體細胞胚 發生, 植物體 再生 및 圃地 移植[1]

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ABSTRACT

Somatic embryo induction, plant regeneration, and field establishment were investigated from tissue cultured winter buds of a 10-year-old tree *Aralia elata*. Embryogenic calli were obtained from cultures of winter buds on MS medium supplemented with 2,4-D. A number of somatic embryos were regenerated from the calli on an embryo induction medium supplemented with 2,4-D and BA. Although abnormal somatic embryos were frequently observed, most of the embryos formed were morphologically normal. All somatic embryos at the later stage of maturity germinated successfully, but only 14% of them could be developed into plantlets on MS basal medium. The plants regenerated from the somatic embryos survived well in the field (survival rates : more than 95%) and have grown normally for three years after transplanting.

Key words : adult tree, somatic embryo induction, plant regeneration

要 約

10년생 두릅나무의 冬芽를 절편으로 體細胞胚를 유도하고 植物體를 再生시켜서 圃地에 활착시키는 실험을 수행하였다. 胚發生 캘러스는 MS 培地에 2,4-D를 첨가하여 誘導되었다. 다량의 체세포배가 MS 배지에 2,4-D와 BA를 동시에 첨가한 조건에서 發生되었다. 배발생 캘러스에서는 기형의 체세포배가 흔히 관찰되었으나 대부분은 全形的인 胚 形態를 보였다. 어뢰형에서 子葉 段階에 이르는 成熟한 體細胞 胚는 모두 發芽가 가능하였으나 완전한 식물체로의 再分化는 MS 기본 배지에서 平均 14% 이었다. 재분화된 식물체는 溫室에서 馴化 後 圃地에서 95% 이상 活着되었고, 移植 後 3년까지 정상적인 生長을 보였다.

INTRODUCTION

Aralia elata is a member of *Araliaceae* and distributes over a large geographic range in Korea, Japan, and China. It is a sunny location deciduous shrub and reaches three-to-four meters in height. Fresh buds and leaves of the tree have long been used as fresh edible vegetables due to their special flavor and nutrition. Although its roots and trunk barks have also been used for traditional folk medicine, the fresh buds and leaves are being

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used as a valuable crop to increase agricultural income sources (Kim, 1996). For a commercial transaction, the fresh buds and leaves have been gathered from wild trees. The practice has caused the continuous erosion of the wild populations. Recently the trees are under intensive cultivation to meet the increasing commercial demand (Jhang et al., 1993). Therefore, it is necessary to supply the propagules for the cultures.

Although seeds may be used to propagate the species, they are difficult to germinate and need a long period for stratification. Rooted cuttings can also be used, but the application is limited to growing season. Furthermore, rooted propagules are very sensitive to damping off disease (Amemiya et al., 1990). Tissue culture systems have shown a great promise with several woody plants. Several tree species have already been successfully propagated by shoot tip and callus cultures. However, not all the woody plants can benefit from the system. For some recalcitrant tree species, somatic embryogenesis may offer an alternative approach. To date, a number of hardwoods have been regenerated via somatic embryogenesis (Chalupa, 1990; Merkle et al., 1989; Merkle et al., 1990, 1995). In *Aralia* species, somatic embryogenesis system using juvenile explants was proven to be an efficient propagation method (Jhang et al., 1993, 1994; Lee and Soh, 1993a,b, 1994; Park et al., 1994). However, there has been no report of successful somatic embryogenesis using mature explants of the species. In the present paper, we report somatic embryogenesis from mature winter buds of 10-year-old *Aralia elata*.

MATERIALS AND METHODS

1. Explant collection and surface disinfection

A 10-year-old (3.5m in height) tree was selected as a stock plant growing at the nursery bed in the Forest Genetics Research Institute, Korea. Branches were collected on April 2, 1994, cut into about 3cm long pieces. After thorns were removed, ten-to-twenty pieces having a bud were put into a 300ml flask and washed thoroughly by a vigorous shaking with tap water containing a few drops of detergent (tween 20). They were disinfested as follows; soaking in 70% ethanol for 5 min, in 2% sodium hypochlorite (NaClO) for 30 min, and finally in sterile water for 5 min. After surface-disinfection, the explants were soaked in sterile distilled water for 30 min.

2. Medium preparation and culture conditions

1) Callus induction

After bud scales were removed with a scalpel, young plumules (about 5mm in length, yellow in color) were isolated and divided into small segments. MS (Murashige and Skoog, 1962) medium was supplemented with three concentrations (0.9, 2.3, or 4.5 μM) of 2,4-D (2,4-dichlolophenoxy acetic acid) were tested for callus induction. Twenty to thirty explants (5 replications per treatment) were placed onto callus inducing medium (CIM) so that the cut surface could contact the medium. The pH was adjusted to 5.7 prior to autoclaving for 20 min at 120℃.

2) Somatic embryo induction

The media were prepared with MS + three different concentrations (0.9, 2.3 or 4.5 μM) of 2,4-D in combination with 0.44 μM benzyladenine (BA) (Table 1). After 7 week culture on CIM, the explants were transferred to embryo induction medium (EIM).

3) Embryo germination and plant regeneration

Embryogenic calli having somatic embryos formed on EIM were subcultured onto plant regeneration medium for embryo germination. MS basal medium either with or without 0.58 μM gibberellic acid (GA_3) was used for plant regeneration. Regenerated plantlets were acclimatized to ambient relative humidity for one month in a controlled environment chamber and then transferred to the green house. Finally the plantlets (about 20cm in height) were transplanted to the field.

4) Culture conditions

All cultures transferred onto fresh medium every 4 weeks and maintained under a 16-hr photoperiod provided by cool white fluorescent lights at 40 μmol m^{-2} s^{-1}. The temperature was constant at 25±2℃.

Table 1. Effect of PGRs on callus and somatic embryo induction from tissue cultured winter buds of *Aralia elata*. The explants were cultured on callus induction medium (CIM) for seven weeks and embryo induction medium (EIM) for three weeks, respectively.

CIM	EIM	No. of calli cultured	No. of calli formed somatic embryos (%)
2,4-D (μM)	BA + 2,4-D (μM)		
0.9	0.44 + 0.9	27	15 (56.0)
	0.44 + 2.3	24	12 (50.0)
	0.44 + 4.5	21	9 (43.0)
2.3	0.44 + 0.9	39	18 (46.0)
	0.44 + 2.3	42	18 (43.0)
	0.44 + 4.5	36	9 (25.0)
4.5	0.44 + 0.9	39	24 (62.0)
	0.44 + 2.3	36	15 (42.0)
	0.44 + 4.5	42	6 (14.0)

RESULTS AND DISCUSSION

All the explants cultured formed calli at the cut surface within one week of culture. Callus was induced in the presence of 2,4-D in all the concentrations tested. It was difficult to discern embryogenic callus from non-embryogenic one in the first 7 weeks of culture, since there was no morphological characteristics unique to each type at the stage. Embryogenic calli could be observed in two weeks after transferring onto EIM. They were yellow, shiny, and slightly compact. This type of calli produced somatic embryos readily (Fig. 1A). Therefore, it was necessary to select the callus type to improve embryogenic potential. The best embryogenic response was obtained by culturing the calli on MS medium supplemented with 4.5 μM 2,4-D in the primary culture and then transferring to the same medium with the combination of 0.44 μM BA and 0.9 μM 2,4-D (Table 1).

Most of the somatic embryos were observed on the surface of the embryogenic callus. Different developmental stages of somatic embryos were simultaneously observed in the embryonic callus, i. e. globular, heart, torpedo, and/or cotyledonary stages (Fig. 1B). Secondary somatic embryos were frequently observed on the primary somatic embryos, especially on the lower part of hypocotyls of the mature somatic embryos. Embryo germination (root elongation and development) was readily achieved on MS basal medium either with or without 0.58 μM GA_3. However, most of the embryos were abnormal. The abnormalties included single cotyledon, horn type cotyledon, and fused cotyledons. Although these abnormal embryos could be germinated, plant regeneration seemed to be suppressed. Normal plant regeneration was achieved when they were cultured on MS basal medium (with the frequency of 14%) and on MS with 0.58 μM GA_3 (with 11% frequency) (Data not shown).

Morphologically normal plantlets were planted to the artificial soil mixture (peatmoss : vermiculrite : peatmoss, 1 : 1 : 1 v/v/v) and acclimatized in the greenhouse. The plantlets survived well and reached 20cm in height after three months (Fig. 1C). The plants were transplanted to field and have grown well for three years (Fig. 1D).

One of major obstacles to the practical application of somatic embryogenesis with woody plants may be low rate of conversion into plants (Merkle, 1995). Various technical as well as chemical treatments have been applied to rescue abnormal somatic embryos and thereby to improve plant regeneration. These include abscisic acid (ABA), osmoticum, desiccation, and cold shock. However, the success rates varied with the species and with the developmental stages of the embryos (Merkle et al., 1990). In our previous experiment, we examined the effect of ABA and BA on both embryo germination and plant regeneration. ABA

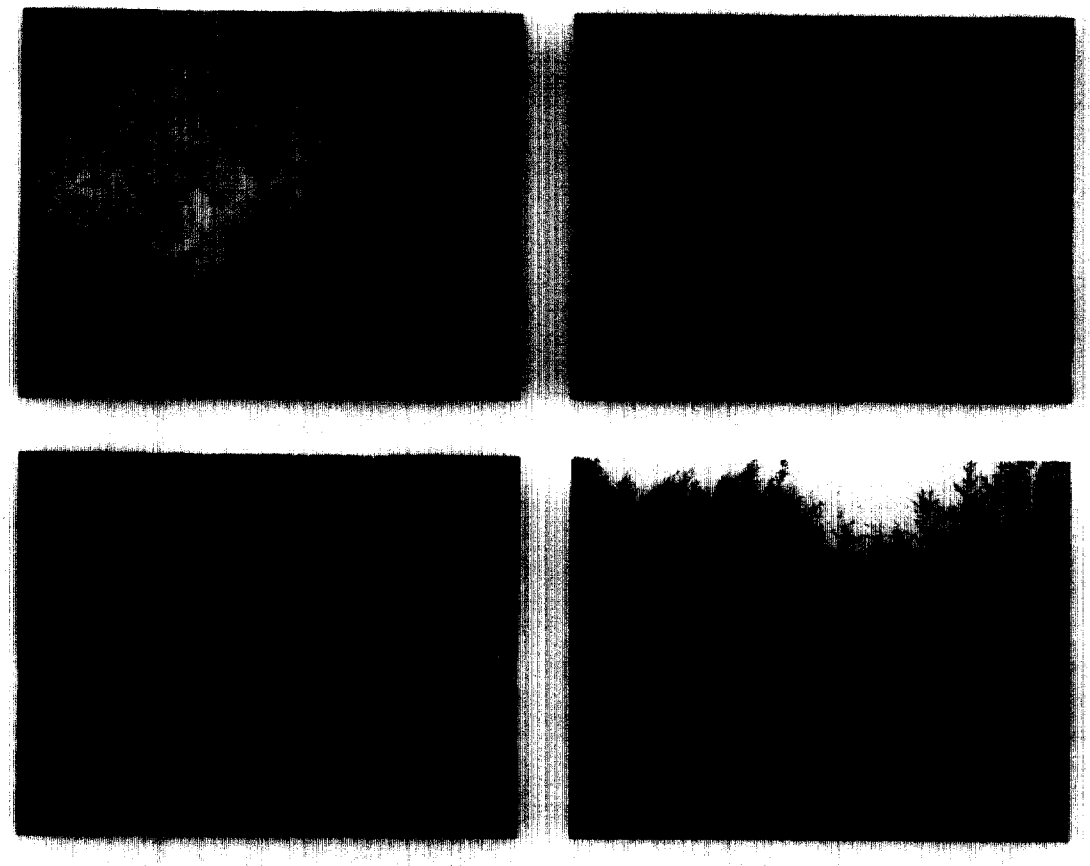

Fig. 1. Somatic embryogenesis and plant regeneration from the winter buds of 10-year-old *Aralia elata*.
A : Early stage of somatic embryos induced from embryogenic callus.
B : Somatic embryos showing various developmental stages on embryo induction medium.
C : Acclimatized plants in the greenhouse.
D : Field growing 3-year-old somatic embryo plants.

appeared to be effective on preventing precocious germination. However, the effect varied greatly with the developmental stages of embryos. When the somatic embryos at the early stage were treated with ABA, root development was retarded and, in the worst case, some of them died. Generally, BA was effective on cotyledon and hypocotyl development. However, germination was suppressed even in the presence of as low as 0.44 μM BA (unpublished data).

In spite of numerous reports on somatic embryogenesis in hardwoods, the data on plant regeneration and field performance are very limited (Merkle, 1995). The present study suggests that somatic embryogenesis in *Aralia elata* using bud explants be an efficient propagation method. We think that developmental stages of the explants and embryogenic callus selection are the key factors to successful somatic embryo induction, maintenance, and plant regeneration.

At present, we are working on the prevention of abnormal somatic embryo formation by changing culture conditions and utilizing cell suspension culture systems. We also started a tissue culture study using other selected individuals that are thornless. The results obtained in the present study suggest that practical application be achieved by somatic embryogenesis systems in *Aralia elata* in the near future. These results also suggest that an efficient regeneration technique be a prerequisite to the practical applications for somatic embryogenesis systems of *Aralia elata*.

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