Effects of Tissue Support and Aeration on the Germination and Growth of *Aralia cordata* Somatic Embryos

Takayoshi Nishihira*, Yoshiaki Hayashi and Kyoko Matsumoto**

Kaneko Seeds Inc., Hashie Institute for Biological Science, Hashie-cho, Isesaki, Gunma 372-0001

Summary

The effects of supporting materials and aeration treatment on the germination and growth of somatic embryos induced from suspension cells of *Aralia cordata* 'Aichi-bozu' were examined.

1. Germination and growth of somatic embryos differed depending on the kinds of supporting materials, e.g. the germination rate after a 4-week culture period was 75.0% for polyester, 56.3% for vermiculite, and 37.5% for agar. Fresh and dry weights of tops of plantlets after a 4-week culture period the polyester support was heavier than these on the others.

2. Seventy percent of the embryos of plot A, which were naturally aerated for the entire 5-week period germinated, whereas 69 % of those in plot B, which were exposed to natural aeration for 2 weeks and forced aeration for another 3 weeks, germinated; only 58 % of the embryos germinated in plot C which were only forced aerated for 5 weeks. All somatic embryos which germinated developed into plantlets. The increment of plant height, fresh and dry weights of plantlets after a 5-week culture period was greater in plot A and B than that in plot C. All plantlets became swollen in plot A but not those in plot B and C. Nevertheless, many normal plantlets developed in plot B. Hence, we found that for the efficient production of the plantlets, polyester was the superior plant support and that high humidity in the first half of a culture period and low humidity in the second half yielded optimum results.

Key Words: aeration treatment, *Aralia cordata*, polyester support, somatic embryos, supporting materials.

Introduction

In a previous paper (Nishihira et al., 1998), we described the effects of different factors and bioreactors on subcultured embryogenic cells on the induction of somatic embryos of *Aralia cordata*. We demonstrated that the highest rate of somatic embryogenesis was obtained from the suspension cells when they were subcultured at 3-week intervals, and that many normal somatic embryos were obtained by using air lift or rotating drum type of bioreactors. Hence, as the next step, factors influencing the germination and growth of normal somatic embryos needed to be investigated. Tsuji et al. (1992) reported that a polyester support was superior to agar for the growth of plantlets derived from carrot somatic embryos, whereas Roberts et al. (1990) affirmed that germination of spruce somatic embryos was suppressed at humidities at or below 81 %, but enhanced above 95 %. Furthermore, Nishimura (1991)

found that the natural aeration treatment was needed at the stage of adventitious shoot induction of tomato and the forced aeration treatment at the stage of adventitious shoot elongation.

From these reports, we surmised that the germination and growth of somatic embryos may be improved by using plant supporting materials and by regulating the environment within the culture vessels. Therefore, in this study we tested the effects of several supporting materials and aeration treatments on the germination and growth of *Aralia cordata* somatic embryos.

Materials and Methods

The somatic embryos were induced from the suspension cells of *Aralia cordata*. 'Aichi bozu' using an air lift type of bioreactor which was aerated at 100 ml·min⁻¹ using an air pump (Nishihira et al., 1998). The suspension cells which were thus mixed induced somatic embryos.

Experiment 1. Germination and growth of somatic embryos using three supporting materials

The materials were: polyester (Low-density polyester; Toyobo Co., Osaka, Japan), vermiculite (small size, 10 kg / bag; Sin Sei Netsuken Kogyo Co.,

Received; February 21, 2000. Accepted; January 29, 2001.

Studies on the micro-propagation of *Aralia cordata* using tissue culture. III.

* Corresponding author: e-mail: hashie@kanekoseeds.co.jp

** Present address: Beppu 3-139, Kumagaya-city, Saitama 378

Fig. 1. Schematic diagram of the culture box.

Box size: height 14 cm, width 31 cm, length 52 cm.

A: Air inlet (size 64 mm²).

B: Air outlet (size 64 mm², There is an air outlet of the same size in opposite side.).

C: Air filter (size 20 cm²).

D: Outlet for culture solution.

E: Basket for supporting materials.

Size of basket: height 7 cm, width 26 cm, length 47 cm.

Tatebayashi, Japan), and agar (TC-5, Lot No.00913; Ina Food Industrial Co., Ina, Japan). Each support was placed in a culture vessel (500 ml) fitted with a teflon-membrane filter (Milliseal, CAT.No.FWMS 01800 ; Nihon Millipore Kogyo Co., Yonezawa, Japan) on the cap (Fig. 3). The weight and size of the polyester support were 2.8 g and 2.4 × 4.8 × 2.4 cm per block, whereas the weight and volume of the vermiculite support were 17.6 g and 100 ml, respectively. The polyester supports of four blocks were placed side by side in the culture vessel. The vessel with the supports and a liquid medium (pH 5.8), contained two-thirds strength MS (Murashige and Skoog, 1962) and supplemented with 30 g · l⁻¹ sucrose, were autoclaved at 120 °C for 15 min. Volumes of the liquid media were 90 ml for polyester and vermiculite supports, 80 ml for agar (80 g · liter⁻¹) support. Eight somatic embryos were placed on supports in a vessel, and ten vessels per treatment which was duplicated. The embryo cultures were kept at 23 °C under a 12-hr photoperiod at a light intensity of 63.3 μmol · m⁻² · s⁻¹ (400–700 nm) fluorescent lamps. The germination rate of the embryos was recorded weekly for 4 weeks after beginning the culture. The leaf number and fresh and dry weights of plantlets were recorded after a 4-week culture period. When the plumules emerged from the embryos, they were considered to have germinated.

Experiment 2. Effects of aeration-treatment on germination and growth of somatic embryos

The stainless steel culture box (Shibata Tekko Co., Ltd., Tokyo, Japan) used for aeration contained 110

Fig. 2. Scheme of three aeration treatments using culture box.

²Air pump (1 kgf · cm⁻²) was used for forced aeration treatments.

Fig. 3. Plantlets grown on three supporting materials after a 4-week culture period.

A: Vermiculite. B: Polyester. C: Agar.

polyester supports placed side by side in a basket (Fig. 1-E). Weight and size of the polyester support per block were the same as blocks used in Experiment 1. The culture medium consisted of two-thirds strength MS liquid medium containing 30 g · liter⁻¹ sucrose. After adjusting the pH of the medium to 5.8, six liters were poured into the culture box and autoclaved at 120 °C for 20 min. Two hundred embryos were placed on the supports in the culture box. The aeration treatment was then performed according to three procedures: (1) natural aeration treatment alone for a 5-week culture period; (2) natural aeration treatment for 2 weeks, followed by forced aeration treatments of 100 ml · min⁻¹ for 2 weeks and 200 ml · min⁻¹ for a week; and (3) only forced aeration treatment of 100 ml · min⁻¹ for 4 weeks, and 200 ml · min⁻¹ for a week. The three procedures designated as plots in A, B, and C, respectively (Fig. 2). For the natural aeration treatment, the atmosphere in the culture box aseptically filtered (AstroPore-FL50; Fuji Photo Film Co., Ltd. Tokyo, Japan) through an air inlet and two air outlets the number of air changes per hour of the culture box = 1.2 hr⁻¹. For the forced aeration treatment, the atmosphere was pumped (1 kgf · cm⁻²; Iwaki Co., Tokyo, Japan) through an aseptic air filter placed on the air inlet of the culture box. Two culture boxes were utilized for each plot and each plot duplicated. Germination rates of the embryos were recorded weekly for 5 weeks. The plant height, leaf number, and

fresh and dry weights of plantlets were recorded at the end of a 5-week culture period. Cultural conditions (day length, temperature and light intensity) were the same as in Experiment 1. Statistical analysis of data was performed by Fisher's PLSD test.

Results and Discussion

Experiment 1. Germination and growth of somatic embryos using several supporting materials

The germination and growth of the embryos differed depending on the kind of supports. The germination rate after 4 weeks of culture was 75.0 % for polyester support, 56.3 % for vermiculite support, and 37.5 % for agar support (Table 1). Top fresh and dry weights of plantlets were also higher on the polyester support than these on the vermiculite and agar supports (Table 2), so that the former was superior for germination and growth of somatic embryos. Tsuji et al. (1992) also reported that properties of polyester supports, especially water retentivity, are closely related to the germination and growth of somatic embryos.

Plantlets on agar support grew the least compared to polyester and vermiculite supports (Table 2, Fig. 3) so that it should not be considered for somatic embryo culture. Agar apparently contains some germination and

growth inhibitors of somatic embryos (Wernicke and Kohlenbach, 1976 and Tyagi et al., 1980).

Experiment 2. Effects of aeration-treatment on germination and growth of somatic embryos

In Experiment 1, since the best results were obtained with polyester, it was used as the supporting material for the rest of the trials.

Tables 3 and 4 list the results of three procedures of aeration treatments. The germination rates in plot A and B after a 5-week culture period of the embryos were 70.0 % and 69.0 %, whereas that in plot C was 58.0 % (Table 3). Therefore, when the natural aeration treatment was given for over the first two weeks, high frequency of germination was obtained. The relative humidity in the natural aeration treatment would be higher than that in the forced aeration treatment because the present experiments were conducted in a room under an atmosphere of low humidity (ca. 50 %). Hence, the germination of *Aralia cordata* somatic embryos may have been stimulated by high relative humidity. Roberts et al. (1990) reported that the highest frequency of germination of spruce somatic embryos occurred when the

Table 1. Effect of supporting materials on germination of *Aralia cordata* somatic embryos obtained by bioreactor.

Support	No. of somatic embryos tested per culture vessel	Germination rate (%) ^z			
		Weeks			
		1	2	3	4
Polyester	8	0	3	75.0	75.0a ^y
Vermiculite	8	0	2	56.3	56.3b
Agar	8	0	2	37.5	37.5c

^zAll somatic embryos germinated on each support developed into plantlets (Table 2).

^yAfter the value of germination rate of somatic embryos which obtained on each support was changed to that of angular transformation, Fisher's PLSD test was performed. Different letters represent significant differences at 1% level (n=20).

Table 3. Effect of aeration period on germination of *Aralia cordata* somatic embryos in a bioreactor^z.

Plot ^y	No. of somatic embryos tested per plant box	Germination rate (%) ^x			
		Weeks			
		1	2	3	5
A	200	0	4	70.0	70.0a ^w
B	200	0	4	69.0	69.0a
C	200	0	2	58.0	58.0b

^zSomatic embryos were cultured with polyester support.

^ySee Fig 2.

^xAll somatic embryos germinated in each plot developed into plantlets, and their growth was shown in Table 4.

^wAfter the value of germination rate of somatic embryos which obtained in each plot was changed to that of angular transformation, Fisher's PLSD test was performed. Different letters represent significant differences at 5% level (n=4).

Table 2. Effect of supporting materials on the development of plantlets from *Aralia cordata* somatic embryos.

Support	No. of leaves per plantlet	Fresh weight (mg/plantlet)		Dry weight (mg/plantlet)	
		Top	Root	Top	Root
Polyester ^z	3.9a ^y	379.0a	—	32.4a	—
Vermiculite	3.8a	235.8b	93.3a	22.8b	6.9a
Agar	3.4a	175.0c	39.3b	18.8c	3.8b

Data were obtained after a 4-week culture period.

^zThe root weights could not be obtained, because roots of plantlets became entangled in the polyester support and were difficult to collect.

^yDifferent letters in the same column represent significant differences by Fisher's PLSD test at 5% level.

Table 4. Effect of aeration period on the development of plantlets derived from *Aralia cordata* somatic embryos.

Plot ^z	Plant height (cm)	No. of leaves per plantlet	Fresh weight of top ^y (mg/plantlet)	Dry weight of top ^y (mg/plantlet)
A ^x	7.0a ^v	4.2a	543.3a	35.2a
B ^w	7.9a	4.3a	538.4a	35.3a
C ^w	5.8b	3.9a	360.2b	29.3b

Data were obtained after a 5-week culture period.

^zSee Fig. 2.

^yIn this test, polyester support was used. Hence, root weights could not be obtained for the reason mentioned in footnote (z) to Table 2.

^xSwelling of all plantlets was observed, especially in the leaves.

^wMorphological traits of plantlets in B and C plots were similar to normal ones which developed from seeds or stubs of *Aralia cordata*.

^vDifferent letters in the same column represent significant differences by Fisher's PLSD test at 5% level.

relative humidity exceeded 95%. Nishimura (1991) demonstrated that many adventitious shoots of tomato were regenerated when calli were exposed to natural aeration during the induction stage. The results of these reports show the same trends as those of our above experiment.

All somatic embryos germinated in the three plots developed into plantlets, which plantlets in plot A and B grew equally tall; those in plot C were significantly shorter (Table 4). Likewise, the top fresh and dry weights of plantlets in plot A and B were not significantly different but heavier than those in plot C. No significant difference in the number of leaves was observed among the plantlets in the three plots although their sizes differed. These results reveal that the increment of plant height, fresh and dry weights of plantlets is promoted when given the natural aeration treatment for over the first two weeks of culture. The morphological traits of plot A plantlets differed from those of the other two plots, becoming more swollen, especially the leaves. Therefore, the natural aeration treatment for long periods may bring about the swelling of plantlets.

Many normal plantlets were eventually obtained in plot B even though the germination rate of somatic embryos was faster in plot B than that in plot C (Table 3, 4, Fig. 4).

We conclude from these results that efficient production of the *Aralia cordata* plantlets is possible through the use of 1) polyester for support 2) high humidity for

Fig. 4. Plantlets developed from *Aralia cordata* somatic embryos after 5 weeks of culture (B plot).

embryo germination during the first two weeks of culture, and 3) low humidity for subsequent growth of plantlets.

Acknowledgement

We wish to thank Dr. Masataka Shimojo of the Faculty of Agriculture, Kyushu University, for his assistance in this research.

Literature Cited

- Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol. Plant.* 15: 473–497.
- Nishihira, T., Y. Hayashi and K. Matsumoto. 1998. Somatic embryos induction from cell suspension culture of *Aralia cordata* using bioreactors. *J. Japan. Soc. Hort. Sci.* 67: 87–92 (In Japanese with English summary).
- Nishimura, S. 1991. Mass propagation of tomato through adventitious bud culture using a large culture box regulating inner environment. *Japan. J. breed.* 41 (Suppl. 2): 232–233 (In Japanese).
- Roberts, D. R., B. C. S. Sutton and B. S. Flinn. 1990. Synchronous and high frequency germination of interior spruce somatic embryos following partial drying at high relative humidity. *Can. J. Bot.* 68: 1086–1090.
- Tsuji, K., M. Nagaoka and M. Oda. 1992. Promotion of the growth of carrot plantlets *in vitro* by controlling environmental conditions in culture vessels. *Acta Horticulturae.* 319: 297–302.
- Tyagi, A. K., A. Rashid and S. C. Maheshwari. 1980. Enhancement of pollen embryo formation in *Datura innoxia* by charcoal. *Physiol. Plant.* 49: 296–298.
- Wernicke, W., and H. W. Kohlenbach. 1976. Investigation on liquid culture medium as a means of anther culture in *Nicotiana*. *Z. Pflanzenphysiol.* 79: 189–198.

培地支持体と通気がウド体細胞胚の発芽と生育に及ぼす影響

西平隆彦・林 義明・松本恭子 *

カネコ種苗(株)波志江研究所 372-0001 群馬県伊勢崎市波志江町 3244

摘 要

培地支持体と通気処理が、ウド‘愛知坊主’の懸だく細胞から誘導した体細胞胚の発芽と生育に及ぼす影響を調査した。

1. 体細胞胚の発芽と生育は、支持体の種類によって異なった。すなわち、培養開始4週間後の発芽率はポリエステルで75.0%, バーミキュライトで56.3%, 寒天で37.5%であった。また、培養開始4週間後の小植物体の地上部の新鮮重と乾物重もポリエステル支持体で大きかった。

2. 培養開始5週間後の発芽率は5週間自然通気のみを行ったA区、2週間の自然通気に引き続いて3週間強制通気を行ったB区、強制通気のみを行ったC区におい

て、それぞれ70.0%, 69.0%, 58.0%となった。発芽したすべての体細胞胚は小植物体へ発達した。培養開始5週間後の小植物体の草丈、新鮮重と乾物重は、C区よりA区とB区で増大した。A区では、すべての小植物体で膨潤化がみられたが、B区とC区ではみられなかった。従って、正常な小植物体はB区で多く得られることが明らかとなった。以上の結果から、小植物体を効率的に生産するためには植物支持体としてポリエステルを用い、培養前半は高湿度、後半は低湿度が適当と推察された。

* 現在: 埼玉県熊谷市別府3-139