식물조직배양학회지 제25권 제1호
Korean J. Plant Tissue Culture
Vol. 25, No. 1, 1~5 (1998)

Anomalous Structure of Somatic Embryos Developed from Leaf Explant Cultures of *Angelica gigas* Nakai

CHO, Duck Yee* · LEE, Eun Kyong[1] · SOH, Woong Young[1]

*Department of Biology, Woosuk University, Chonbuk, 565-701, Korea: and
[1]Department of Biological Sciences, Chonbuk National University, Chonju, 561-756, Korea. *Corresponding author.

This study describes the effect of the growth regulators such as 2,4-D and BA, on the structural abnormalities of somatic embryos derived from leaf explants of *Angelica gigas* Nakai. Also, the relationship between the cotyledon number of a somatic embryo and its germinability is explored. Embryogenic calli were selected from calli formed on explants cultured on MS solid basal medium supplemented with 0.5 mg/L 2,4-D, 1 mg/L 2,4-D, 1 mg/L 2,4-D plus 0.1 mg/L BA, and 1 mg/L 2,4-D plus 0.5 mg/L BA. Cotyledonary abnormalities were observed in somatic embryos which were developed from embryogenic calli cultured on MS medium containing 1mg/L 2,4-D for 8 weeks and then subcultured on 2,4-D free MS medium for 3 weeks. The frequency of abnormalities was as follows: 22.8% one cotyledon, 42.5% two cotyledons, 16.8% three cotyledons, 7.8% four cotyledons, 1.8% five cotyledons, and 8.2% jar shaped cotyledon. In addition, ABA treatment indicated an improvement of the somatic embryo with normal cotyledon (65.3%). ABA was important role to the high production of normal somatic embryos. Two cotyledon embryos showed germinability 77.8%. However, the germinability of somatic embryos with anomalous cotyledons was prominently low: One cotyledon, 62.5%; three cotyledons, 43.3%; four cotyledons, 60%; five cotyledons, 50% and jar shaped cotyledon, zero%. Thus, germinability was essentially, inversely proportional to cotyledon number.

Key words: ABA, *Angelica gigas*, cotyledon, somatic embryogenesis, germinability, jar shape

Research on somatic embryogenesis has contributed to advancements in plant biotechnology such as artificial seed development. The plant regeneration rates of somatic embryos formed from tissue cultures varies greatly depending on the plant species, and it has been clarified that the structure of somatic embryos is closely related to the plant regeneration rate (Soh, 1996: Soh et al., 1996). Although it is known that both zygotic and somatic embryos have identical structure, there are morphological differences between them in many species (Ammirato, 1977: Soh, 1993: Wetzstein and Baker, 1993: Lee and Soh, 1994: Cho and Soh, 1995: Soh et al., 1996). In case of *Aralia cordata*, the development of embryos with multiple and jar shaped cotyledons occurred most frequently when cultured continuously on medium containing cytokinins (Lee and Soh, 1993a), on 2,4-D-containing medium (Lee and Soh, 1993b) or on ABA-containing medium (Lee and Soh, 1994).

In both *Bupleurum falcatum* and *Daucus carota*, the plant regeneration rate is inversely proportional to the number of cotyledons in embryos with anomalous cotyledons (Cho and Soh, 1995: Soh et al., 1996), while it is identified that, in *Aralia cordata*, the rate was in direct proportion to the number of cotyledons (Lee and Soh, 1993a). Thus, the structure of somatic embryos plays a significantly important role in the plant regeneration rate of somatic embryos. This study is aimed to contribute to the enhancement of the production and regeneration rates of somatic embryos using plant growth regulators such as ABA.

MATERIALS AND METHODS

The leaves of *Angelica gigas* Nakai, were collected in the area around Kumsansa temple located in Kimje City, Chollabukdo, Korea. The collected leaf explants were first sterilized in 70% ethanol for 1 minute, disinfected with 1% sodium hypochlorite solution for 15 minutes, and then rinsed three or four times with sterile distilled water. For initiation

of callus, surface-sterilized leaf segments (3 × 3 mm) were placed on MS basal medium (Murashige and Skoog, 1962) supplemented with various concentrations of 2,4-D (0.1, 0.5, 1, or 2 mg/L) alone, or in combination with BA (0.1, 0.5, 1, or 2 mg/L), sucrose at 30 g/L and agar at 0.8%. The pH was adjusted to 5.8 prior to autoclaving at 121°C for 15 minutes and medium dispensed into 100ml Elerenmeyer flask with 40ml in each. The cultures were maintained under cool white fluorescent light 46 μmol $m^{-2}s^{-1}$ with 16/8 light and dark cycle at 25 ± 1°C. Calli were induced from leaf explants on the MS medium supplemented with 2,4-D and BA after 8 weeks culture. In order to induce normal somatic embryo with two cotyledons, the embryogenic calli were transferred onto MS basal medium containing various concentrations (0, 0.04, 0.2, or 1 mg/L) of ABA for three weeks.

Germination was confirmed by the appearance of the primary leaf after rooting and germination frequency was measured 4 weeks later. The characteristics of somatic embryos were observed at least 100 in each trial with three repeated.

RESULTS

After 8 weeks culture on MS medium with various concentrations (0.1, 0.5, 1, or 2 mg/L) of 2,4-D alone or combination with BA (0.1, 0.5, 1, or 2 mg/L), the explants formed pale yellow and green calli. The pale yellow calli were formed on the 2,4-D only medium (Fig. 1A), and green calli on medium treated with 2,4-D and BA in combination. After 8 weeks of culture, embryogenic calli were transferred onto MS basal medium in order to induce somatic embryos. The somatic embryo was formed on the MS medium supplemented with 0.5 and 1 mg/L 2,4-D alone, or combination with BA (0.1, 0.5 mg/L) (Table 1). The white globular embryos appeared in the shapes of beads (Fig. 1B, arrows) on these medium. These embryos matured into cotyledon stage embryos through successive developmental stages (Fig. 1C).

Somatic embryos with anomalous cotyledons such as embryos with jar shape, one cotyledon, or four cotyledons, (Fig. 1D, E, G) were formed in addition to embryos with normal two cotyledons (Fig. 1F). When cultured on MS medium supplemented with 1 mg/L 2,4-D, diverse cotyledon abnormalities appeared as follows: 42.5% normal somatic embryos with two cotyledons, 22.8% one cotyledon embryos, 16.9% three cotyledon embryos, 7.8% four cotyledon embryos,

Table 1. Effects of 2,4-D and BA on somatic embryogenesis from leaf explant cultures of *Angelica gigas* Nakai.

BA (mg/L)	2, 4-D (mg/L)				
	0	0.1	0.5	1	2
0	-	+	+++	+++	+
0.1	-	-	+	++	+
0.5	-	-	+	++	+
1	-	-	+	+	+
2	-	-	-	-	-

+++:excellent, ++: very good, +: good, -: bad
Each explant was cultured on MS agar medium supplemented with various concentrations of 2,4-D alone or combination with BA. Calli were induced on leaf explants after 8 weeks of culture. Embryogenic calli were selected from the calli and cultured on MS basal media for 3 weeks of culture in order to produce somatic embryos.

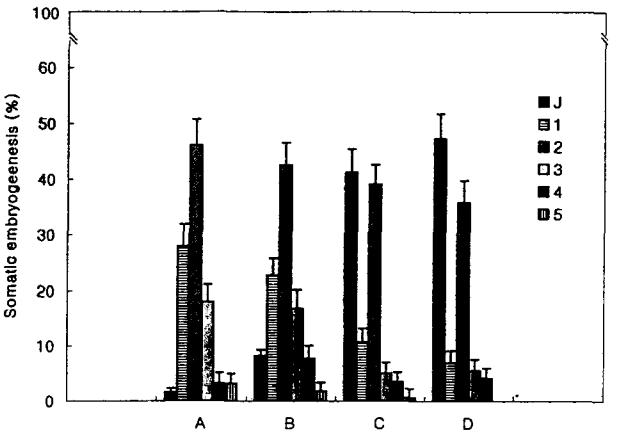

Figure 2. The frequency of somatic embryos with different cotyledon pattern of *Angelica gigas* Nakai. The somatic embryos formed on MS basal medium for 3 weeks after transfering from MS medium supplemented with 0.5 mg/L 2,4-D (A), 1 mg/L 2,4-D (B), 1 mg/L 2,4-D + 0.1 mg/L (C) and 1 mg/L 2,4-D + 0.5 mg/L BA (D). J: Jar shaped, 1: one cotyledon, 2: two cotyledons, 3: three cotyledons, 4: four cotyledons, 5: five cotyledons. Each treatment consisted of 100 embryos.

Table 2. Effect of ABA on the cotyledon number of somatic embryos from leaf explant cultures of *Angelica gigas* Nakai.

ABA (mg/L)[a] \ Cotyledon types(%)	Jar	2	3	4	5
0.00	8.2±0.89	22.8±2.36	42.5±4.31	16.9±1.71	7.8±0.79	1.8±0.19
0.04	3.9±0.31	15.5±1.78	65.3±5.25	10.2±0.12	2.5±0.34	2.6±0.34
0.2	3.1±0.29	19.1±2.10	63.0±5.06	8.8±0.73	2.9±0.27	3.1±0.31
1.0	6.6±0.65	27.3±2.68	42.2±4.20	18.0±1.80	4.4±0.51	1.5±0.15

[a]Cultures were treated with 1 mg/L 2,4-D for 8 weeks and were transferred to MS medium containing three levels ABA for 3 weeks. The data represent the mean ± SE of three replicated experiments. They were obtained from the count of at least 100 embryos in each treatment.

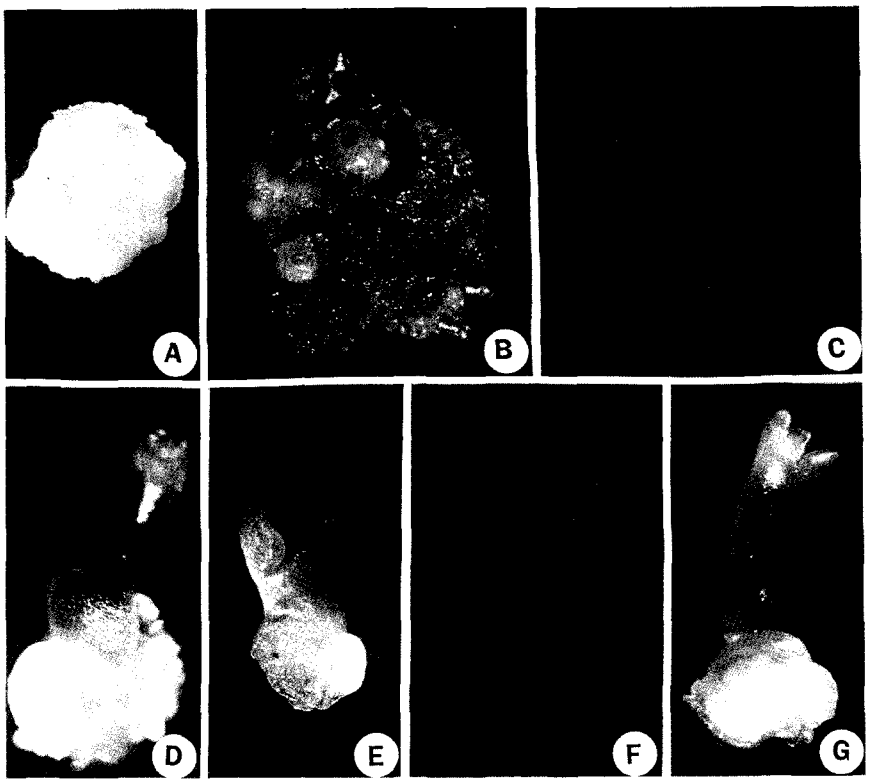

Figure 1. Induction of somatic embryogenesis from leaf explant cultures of *Angelica gigas* Nakai. A: non embryogenic callus with pale yellowish, B: globular stage embryo (arrows), C: various types of embryo formation, D: Jar shaped embryo, E: one cotyledon, F: two cotyledons, G: four cotyledons.

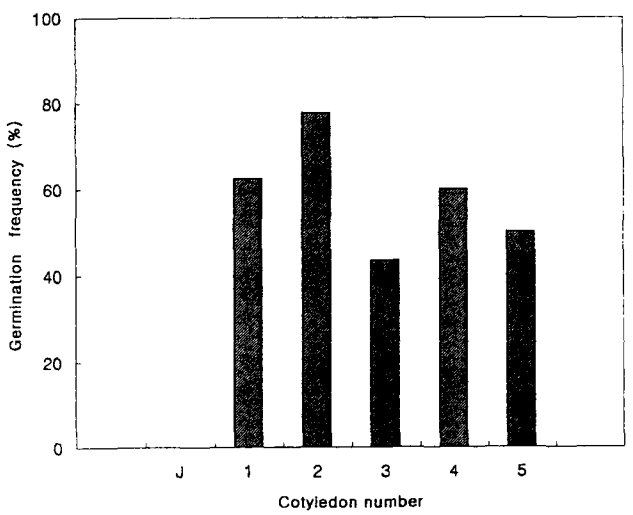

Figure 3. Germination frequency (%) of somatic embryos formed from callus cultures of *Angelica gigas* Nakai. The leaf explant was cultured on MS agar medium supplemented with 1 mg/L 2,4-D for 8 weeks and then subcultured on hormone free MS medium for 3 weeks. Data were collected from three replicates with 100 embryos each. J: jar shaped embryo, 1: one cotyledon, 2: two cotyledon, 3: three cotyledon, 4: four cotyledon, 5: five cotyledon. Jar shaped somatic embryo did not germinate only rooting appeared.

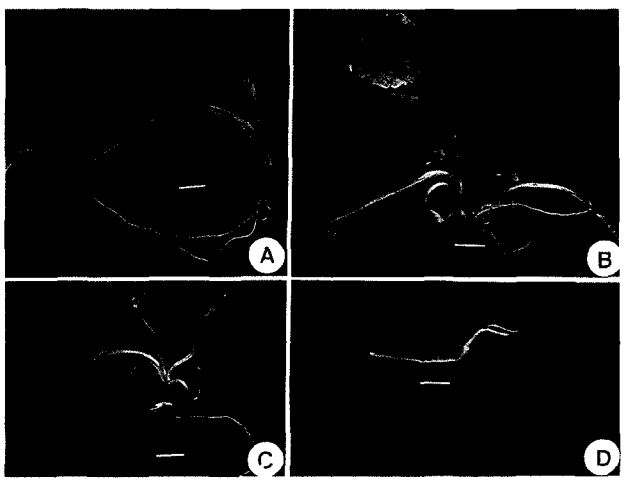

Figure 4. Germination of *Angelica gigas* L. somatic embryos with various typed cotyledon. A: one cotyledon, B: normal somatic embryo with two cotyledon, C: three cotyledons, D: jar shaped embryo develops root and enlarged cotyledon but did not develop shoot. Scale Bars represent 8 mm.

1.8% five cotyledon embryos, and 8.2% jar shaped embryos. On the MS medium supplemented with 1 mg/L 2,4-D + 0.5 mg/L BA jar shaped embryos appeared at a high frequency of 47.2%, and one, two, three and four cotyledon embryos were 7.1%, 35.7%, 5.7%, and 4.3% respectively (Fig. 2). When the embryogenic calli were transferred on MS basal medium containing 0.04 mg/L ABA for 3 weeks, the frequency of normal somatic embryos with two cotyledons was 65.3%. Somatic embryos with anomalous cotyledons was

prominently low: jar type 3.9%, one cotyledon, 15.5%: three cotyledons, 10.2%, four cotyledons, 2.5% and five cotyledons, 2.6%. But MS medium supplemented with higher concentration of ABA (1 mg/L) had low frequency of somatic embryos with two cotyledons (Table 2). Therefore optimal concentration of ABA on the enhanced production of normal somatic embryos was 0.04 mg/L.

The rate of germination differed depending on the cotyledonary abnormality. The rate for normal somatic embryos with two cotyledons was 77.8%, while somatic embryos with one, three, four and five cotyledons were 62.5%, 43.3%, 60%, and 50% respectively, and jar shaped embryos did not show any germinability at all (Fig. 3, 4).

DISCUSSION

When leaf explants of *Angelica gigas* were incubated on MS medium supplemented with 2,4-D (0.5, 1 mg/L) alone or in combination with BA (0.1, 0.5 mg/L) respectively, the production of somatic embryos with two cotyledons in each medium was lower than 50%. And the rate of somatic embryos with jar shaped embryos on medium supplemented with the combination of 1 mg/L 2,4-D with BA(0.1 or 0.5 mg/L) was higher than on medium containing 2,4-D alone. In embryogenic calli of melone were cultured on BA-containing medium, jar type or horn type embryos appeared higher than normal embryo with two cotyledons at three times (Choi et al., 1994a). Thus it is suggested that BA effects abnormal cotyledon development of somatic embryos. This is similar to reports In *Aralia cordata* and caraway embryos where somatic embryos with anomalous cotyledons were formed at a high frequency on medium supplemented with BA (Ammirato, 1977: Lee and Soh, 1993a). The frequency of abnormal embryos in the present experiment is significantly higher than in the case of *Bupleurum falcatum*, where the frequency of somatic embryos with anomalous cotyledons was 35% (Cho and Soh, 1995), and in case of Daucus carota of 37% (Soh et al., 1996).

When embryogenic calli were transferred on MS medium supplemented with relatively lower concentrations of ABA (0.04, or 0.2 mg/L) for three weeks, they were enhanced production of normal somatic embryos with two cotyledons (65.3% and 63.0%). Thus, it is suspected that variation in the development of cotyledons in somatic embryos is caused by in an imbalance of plant growth regulators in culture medium.

When globular embryos of *Aralia cordata* was incubated continuously on MS medium with 2,4-D (Lee and Soh, 1993a), or when immature zygotic embryos of Glycine max L. were cultured in 0.5-4 mg/L 2,4-D, the number of anomalous cotyledons increased (Lee and Soh, 1994: Choi et al., 1994b). During the process of somatic embryogenesis, if an inhibitor of auxin transport was treated to the ovule culture, jar shaped somatic embryos were formed because of an uneven distribution of endogenous auxins (Liu et al., 1993).

In *Codonopsis lanceolata* and *Glycine max*, normal somatic embryos with two cotyledons were produced at low concentrations (5-20 mg/L) of sucrose (Choi et al., 1994b,c). Thus, the production of abnormal cotyledonary embryos might be affected greatly not only by plant growth regulators, but also by the concentration of sucrose (Kageyama et al., 1990: Komatsuda, 1990). Therefore, the selection of culture medium composition is very important in order to lower the frequency of abnormal somatic embryogenesis. Detail examination on the physical and chemical factors affecting the medium is greatly required.

Cotyledonary variation in somatic embryos is closely related to the germination frequency: normal embryos with two cotyledons were 77.8%, but somatic embryos with anomalous cotyledons were very low in *Angelica gigas*. In *Bupleurum falcatum*, the germination rate of somatic embryos with two cotyledons was 80%, but the rate of somatic embryos with multiple cotyledons was quite low (Cho and Soh, 1995). The number of cotyledons was inversely related the regeneration rate with in *Codonopsis lanceolata* (Choi et al., 1994b), *Vitis vinifera* (Isabelle et al., 1993), and *Daucus carota* (Soh et al., 1996) as well. In contrast, *Aralia cordata* showed a different regeneration pattern, where the frequency of regeneration was proportional to the number of cotyledons (Lee and Soh, 1994).

In addition, it was observed that, no germination of the jar shaped embryos was observed in plants such as *Codonopsis lanceolata*, *Bupleurum falcatum*, *Daucus carota*, and *Aralia cordata* (Isabelle, 1993: Lee and Soh, 1994: Choi et al., 1994: Cho and Soh, 1995: Soh et al., 1996). In *Daucus carota*, it was observed that root growth occurred by expansion in the cotyledonary portion which was not accompanied by shoot formation (Soh et al., 1996). This loss of normal germinability in jar shaped cotyledon embryos is considered to arise from the lack of shoot development due to abnormalities in the activity of the shoot apical meristem (Nickle and Yeung, 1993: Soh et al., 1996). Therefore, it is necessary to investigate the inhibitive culture conditions that affect the

occurrence of jar shaped cotyledons (Cho and Soh, 1995).

적 요

당귀(*Angelica gigas* Nakai)의 잎절편유래 체세포배의 비정상자엽구조에 관한 2,4-D와 BA의 영향에 대하여 또한 체세포배의 자엽수와 발아와의 관계에 대하여 기술하였다. 배발생능 캘러스는 0.5 및 1 mg/L 2,4-D와 1mg/L 2,4-D+ 0.1 mg/L BA, 1mg/L 2,4-D+ 0.5 mg/L BA가 첨가된 MS기본배지에 치상한 외식편 에 형성된 캘러스로부터 선발하였다. 1mg/L 2,4-D첨가 MS고형 기본배지에서 8 주간 배양후 2,4-D가 제거된 배지에서 3주간 배양하여 체세포배 발생과 비정상적인 체세포배의 자엽발생을 관찰하였다. 이상자엽의 빈도는 정상인 2개의 자엽을 갖는 체세포배는 42.5%인데 반하여 1개의 자엽에서 22.8%, 3개의 자엽을 갖는 체세포배에서는 16.8%, 4개의 자엽에서는 7.8%, 5개의 자엽배에서는 1.8%와 주발형 자엽배는 8.2%이었다. 또한 ABA를 처리하면 2개의 자엽을 갖는 체세포배는 65.3%로 향상되었다. ABA 는 정상적인 체세포배의 높은 생산에 중요하였다. 2개의 정상적인 자엽을 갖는 체세포배의 발아율은 77.8%이었으나 비정상적인 자엽을 갖는 체세포배의 발아율은 대단히 낮아서 1개, 3개, 4개, 5개 및 주발형의 자엽배을 갖는 체세포배에서는 62.5%, 43.3%, 60%, 50% 및 0%이었다.

ACKNOWLEDGEMENT - This study was supported by the Basic Science Research Institute Program (BSRI-95-4427), Ministry of Education, Republic of Korea

LITERATURES CITED

Ammirato PV (1977) Hormonal control of somatic embryo development from cells of caraway. Interaction of abscisic acid, zeatin and gibberllic acid. Plant Physiol **59**: 579-586

Cho DY, Soh WY (1995) Morphological observation of somatic embryogenesis in leaf explants cultures of *Bupleurum falcatum* L. Korean J Plant Tisssue Culture **22**: 291-297

Choi PS, Soh WY, Cho DY, Liu JR (1994a) High frequency somatic embryogenesis and plant regenerationin seedling explant cultures of melon (*Cucumis melo* L.) *J.* Plant Tissue Culture **21**: 1-6

Choi PS, Soh WY, Cho DY, Liu JR (1994b) Somatic embryogenesis in immature zygotic embryo cultures of Korean soybean (*Glycine max* L.) cultivars and effect of 2,4-dichlorophenoxy acetic acid on somatic embryo morphology. Korean J Plant Tissue Culture **21**: 7-13

Isabelle GT, Mauro M.C, Sossountzov L, Deloire A (1993) Arrest of somatic embryo development in grapevine: histological characterization and the effect of ABA, BAP and Zeatin in stimulating plantlet development. Plant Cell Tissue Org Cult **33**: 91-103

Kageyama C, Komatsuda T, Nakajima K (1990) Effects of sucrose concentration on morphology of somatic embryos from immature soybean cotyledons. Plant Tissue Culture Letters **7**: 108-110

Komatsuda T (1990) Ability of soybean (*Glycine max* L. Merr.) genotypes to produce somatic embryos on a medium containing a low concentration of sucrose. Japan J Breed **40**: 371-375

Lee KS. Soh WY (1993a) Somatic embryogenesis and structural aberrancy of embryos in tissue cultures of *Aralia cordata* Thunb. Korean J Plant Tissue Culture **20**: 77-83

Lee KS, Soh WY (1993b) Effects of cytokinins on the number of cotyledons of somatic embryos from cultured cells of *Aralia cordata* Thunb. Korean J Plant Tissue Culture **20**: 171-175

Lee KS, Soh WY (1994) Effects of ABA on abnormal somatic embryogenesis in tissue cultures of *Aralia cordata* Thunb. Korean J Plant Tissue Culture **21**: 287-291

Liu CM, Xu ZH, Chua NH (1993) Auxin polar transport is essential for the establishment of bilateral symmetry during early plant embryogenesis. The Plant Cell **5**: 621-630

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant **15**: 473-497

Nickle TC, Yeung EC (1993) Failure to establish to functional shoot meristem may beacause of conversion failure in somatic embryos of *Daucus carota* (Apiaceae). Amer J Bot **80**: 1284-1291

Soh WY (1993) Developmental and structural diversity of generated plants in cell and tissue cultures. In Proc. 7th Symposium on Plant Biotechnology, Molecular Approach to Plant Cell Differentiation. Bot Soc Korea pp. 1-35.

Soh WY (1996) Germinability and cotyledon structure of somatic embryos in some dicotyledonous plants. Proceedings of 2nd Asia-Pacific Conference on Plant Cell and Tissue Culture, Beijing pp. 51-59.

Soh WY, Cho DY, Lee EK (1996) Multicotyledonary structure of somatic embryos formed from cell cultures of *Daucus carota* L. J Plant Biol **39**: 71-77

Wetzstein HY, Baker CM (1993) The relationship between somatic embryo morphology and conversion in peanut (*Arachis hypogaea* L.). Plant Sci **92**: 81-89

(1997년 10월 17일 접수)