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Production of Somatic Embryos in *Oenanthe javanica* (BL.) DC.

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미나리의 體細胞 胚 生産 硏究

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This study was carried out to establish a mass production of normal somatic embryos of *Oenanthe javanica* (BL.) DC. including examination of nitrogen and sucrose sources, and ABA concentration. Embryogenic cell clumps and embryos were formed on the MS medium devoid of growth regulators. Proliferation of embryogenic cells and clumps was enhanced by 2,4-D. Meanwhile embryo growth and development occurred on the media containing NAA and IBA. Growth of embryos was generally good in the media containing both 20 mM KNO_3 and 20 mM NH_4NO_3. The rate of shoot forming embryos was higher on the media containing only 20 mM NH_4NO_3 than on the former. Addition of sucrose at 3 - 6% enhanced the embryo development, and normal embryos with short hypocotyl was observed on the medium containing 10 μM ABA. Embryogenic cell clumps or globular embryos, when transferred to MS solid media devoid of growth regulators, developed into mature embryos and then into plantlets which had entire primary leaves like zygotic seedlings.

Key words: somatic embryos, mass propagation, *Oenanthe javanica*

In vitro somatic embryogenesis has a great merit in plant propagation when compared to *in vivo* adventive or asexual embryogenesis in ovular tissue. In order to obtain normal somatic embryos as propagules or artificial seeds, it is necessary to understand developmental response of somatic embryos to growth regulators, nutrients and culture conditions (Kim et al., 1988: Kitto and Janick, 1985a, b: Redenbaugh et al., 1987). *O. javanica* (BL.) DC. has been consumed as healthy and fresh vegetable in Korea for a long time and the consumption and production has been consistently increasing (Kim, 1986: Yang, 1985). Kim (1986) suggested that for intensive culture system under the structures including hydroponics, propagation by seeds can be an effective method to obtain uniform planting material free of virus. However, use of seeds is unpractical due to the difficulty in obtaining a large amount of seeds. In conventional cultivation of *O. javanica* (BL.) DC. cuttings and divisions has been used to obtain planting material. Due to the heterogeneity of the planting material, uniform plant establishment is generally hard to attain. Therefore, the present study was carried out to establish an efficient culture system for production of somatic embryos of *O. javanica* (BL.) DC. and raising them into plantlets as planting material.

MATERIALS AND METHODS

The embryogenic calli (ca. 100 mg) obtained from the primary culture of zygotic pro-embryos or *In vitro* petiole segments (Koh, 1995a, b) were transferred to 100 ml Erlenmeyer flasks filled with 50 ml MS liquid basal medium. The suspension cultures were subcultured every 10 to 14 days

in the same media with 100 - 110 rpm. In order to find out the effects of growth regulators, embryogenic cell clumps were cultured in the liquid media containing BA in combinations with NAA, 2,4-D or IBA. In order to find the effects of nitrogen source, NH_4NO_3 and KNO_3 were added to the MS liquid media in combinations at 4 concentration levels (0, 10, 20 and 40 mM).

In order to enhance the development of normal mature embryos, the globular embryos, collected by sieving the suspension cultures through 0.5 mm × 0.5 mm sieve, were cultured on the MS liquid media supplemented with ABA and carbohydrates: the concentrations of ABA were 1, 3, 6 and 10 μM. Sucrose or glucose were added at 0, 3, 6, 9 and 12%.

Somatic embryos were transferred to MS basal medium solidified with 0.8% agar. The normal plantlets derived from embryos were transferred to perlite and then to the outdoor soils.

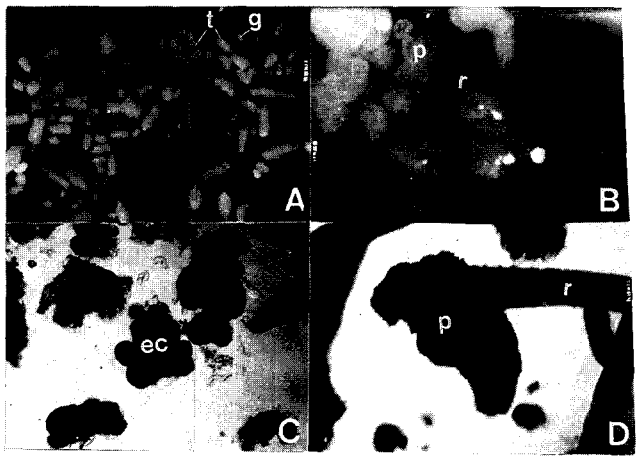

Figure 1. Development of globular embryos of *O. javanica* (BL.) DC. cultured in liquid MS media containing various growth regulators.
A: Embryo development in the medium free of growth regulators. Embryos developed upto globular embryos (g) and torpedo stages (t) was normal but those at advanced stage had elongated roots. B: Development of abnormal embryos in the media containing 1.0 mg/L NAA. The embryos had well developed radicle (r) but clumped plumule (p). C: Continuos proliferation of embryogenic clumps (ec) on the media containing 1.0 mg/L 2,4-D. D: Development of abnormal embryos in the media containing 1.0 mg/L IBA. The embryos had well developed radicle (r), but poorly developed plumule (p).

RESULTS AND DISCUSSION

Development of somatic embryos in suspension culture

A number of embryos were produced on MS basal liquid medium under dim light. Embryogenic callus continuously proliferated embryogenic cell clumps, which developed proceeded into globular, torpedo and unusually elongated embryos. Globular embryos developed normally into torpedo embryos, which failed to develop into normal cotyledonely embryos. The abnormal embryos had elongated hypocotyl and underdeveloped cotyledon but well developed roots (Figure 1A). The embryos became greenish in color as soon as they grew into mature embryos. Most of the embryogenic cells developed into embryogenic cell clumps in the auxin free medium. Embryogenic cell clumps became large in size, and the interior parts of clump became a main sources of non-embryogenic cells in the suspensions. The radicles or root fragments of the developing embryos also seemed to produce non-embryogenic cells.

The embryogenic cell clumps rarely proceeded embryogenesis but continually produced embryogenic clumps when cultured in the liquid media containing 2,4-D (Figure 1C). Growth of radicle and plumule of the embryos was repressed by 2,4-D even at a low concentration (0.1 mg/L), and embryo development did not occur at high concentrations of 2,4-D above 0.5 mg/L (Table 1). Embryogenic cell clumps of carrot proliferated well, but development of embryos did not occurred in the liquid media containing 2,4-D, while, embryos development occurred rapidly after transfer to the liquid media devoid of 2,4-D (Street and Withers, 1974: Halperin and Jensen, 1967). Development of somatic embryo in *O. javanica* (BL.) DC. showed a similar pathway to those in carrot: Embryo development did not occur in the liquid medium containing 2,4-D, while it did on the media lacking 2,4-D.

The media containing NAA produced less embryogenic cells than those containing 2,4-D (Figure 1B). However, the number of embryogenic cell clumps increased as NAA concentration increased. Embryos developed only upto the torpedo stage in the media containing NAA less than 0.5 mg/L. Root development occurred in the media containing NAA at various levels but shoot development did not occur in the media containing NAA above 1.0 mg/L. The plumules of the embryos turned into embryogenic clumps like a string of beads, instead of forming shoots (Figure 1B).

The globular embryos cultured in the media containing IBA produced less embryogenic cells than those cultured in the media containing 2,4-D or NAA. Development of somatic embryos progressed up to torpedo stage in the media

Table 1. Effects of 2,4-D, NAA, IBA and BA on the embryo development in suspension culture of globular embryos of *O. javanica* (Bl.) DC. in MS media under dim light for 3 weeks.[a]

BA (mg/L)	Auxin (mg/L)	Single cells[b]	Globular embryos[c]	Torpedo embryos[d]	Mature embryos[d]	Root[e]	Shoot[f]
	2,4-D						
0.0	0.1	+++	++	22 ± 5	86 ± 21	L+N+	+
	0.5	++++	++	10 ± 2	10 ± 1	-	-
	1.0	++++	+	0	0	-	-
	2.0	+++++	+	0	0	-	-
0.1	0.1	++	+	10 ± 3	12 ± 1	-	-
	0.5	+++	+	0	0	-	-
	1.0	+++	+	0	0	-	-
	2.0	++++	+	0	0	-	-
	NAA						
0.0	0.1	++	++	35 ± 7	55 ± 12	L++N+	++
	0.5	+++	+++	42 ± 6	85 ± 11	L++N++	+
	1.0	+++	+++	36 ± 6	45 ± 8	L+++N++	+
	2.0	++++	++	15 ± 1	11 ± 4	L++++N++	-
0.1	0.1	+	++	32 ± 4	55 ± 21	L+N+	++
	0.5	++	++	45 ± 2	60 ± 14	L++N+	+
	1.0	+++	+++	43 ± 8	70 ± 16	L++N++	+
	2.0	+++	++	45 ± 11	20 ± 5	L+++N+	-
	IBA						
0.0	0.1	+	++	42 ± 14	0	L++++N+	++
	0.5	++	++	41 ± 11	0	L++++N+	+
	1.0	+++	+++	36 ± 2	0	L+++N+	-
	2.0	+++	++	17 ± 4	0	L+N+	-
0.1	0.1	+	++	33 ± 3	14 ± 1	L+++N+	+
	0.5	+++	+	25 ± 8	0	L++N+	-
	1.0	+++	++	24 ± 3	0	L++N+	-
	2.0	+++	++	15 ± 1	0	L+N+	-
0.0	0.0	++	++++	87 ± 12	201 ± 30	L+++N++	+++
0.1	0.0	++	++	42 ± 2	25 ± 4	L++N+	+++

[a] Data collected from 3 replicates of 100 ml flask. [b] and [c] +: few, ++ : a few, +++: medium, ++++: many and ++++: very many. [d] Number of embryos± S.D.
[e] L+: very short, L++: short, L+++: long, and L++++: very long, N+ : a few, N++ : many. [f] +: very poor, ++: poor and +++: medium.

containing IBA below 0.5 mg/L, but did not progressed into mature embryos (Figure 1D). The plumule ends of embryos became embryogenic clumps in the media containing 2.0 mg/L IBA. Root growth was especially good in the media containing IBA less than 1.0 mg/L. Root development from the somatic embryos, in terms of number and length, was better on the media containing IBA than those on those containing NAA or 2,4-D (Figure 1D).

Embryo development, in terms of number and fresh weight, was better on the media containing both 20 mM KNO_3 and NH_4NO_3 than those on the media containing only one kind. Development of globular embryos was suppressed on the media containing 40 mM KNO_3 or 40 mM NH_4NO_3. Embryo development was good in the media containing either

Table 2. Effects of nitrogen source (NH_4NO_3 and KNO_3) in MS liquid media on the fresh weight (g) of embryogenic cell clumps and embryos of *O. javanica* (Bl.) DC. under dim light for 3 weeks.[a]

KNO_3 (mM)	NH_4NO_3(mM) 0	10	20	40
0	0.38	0.84	1.03	0.79
10	0.65	4.00	3.47	3.34
20	1.41	6.75	7.69	0.34
40	0.66	0.82	0.39	0.33

[a]100 mg fresh weight of embryogenic cell clumps was inoculated in MS liquid media containing the nitrogen sources in various concentrations. Data collected from 3 replicates of 100 ml flask.

Table 3. Effects of nitrogen source (NH_4NO_3 and KNO_3) in MS liquid media on the growth and development of shoot, hypocotyl and root of the embryos of *O. javanica* (Bl.) DC. for 3 weeks.[a]

Parts	KNO_3 (mM)	NH_4NO_3 (mM) 0	10	20	40
Shoot	0	-	++	+++	++
	10	-	+	++	+
	20	+	+	+	+
	40	-	-	-	-
Hypocotyl	0	-	+	+	+
	10	+	++	+	+
	20	+	+++	+++	+
	40	-	++	++	++
Root	0	+	+	+	+
	10	+	++	++	+
	20	++	++	++	+
	40	++	++	++	+

[a]S: shoot, H: hypocotyl and R: root, and -: none, +: a little, ++: medium and +++: good development, respectively.

20 mM KNO_3 and 10 mM NH_4NO_3 or 20 mM KNO_3 and 20 mM NH_4NO_3. The number of shoots producing embryos was higher on the media containing 20 mM NH_4NO_3 than those containing either only 20 mM KNO_3 or 20 mM KNO_3 and 20 mM NH_4NO_3 (Table 2, and 3). This data indicates that there may be a positive correlation between intra-cellar NH_4^+ concentration and embryo development. Ammirato (1969) reported that the cotyledon growth in the embryos was fostered by nitrogen, particularly in the form of ammonium, even though fresh weight or growth rate was not better than those in the media containing both NH_4NO_3 and KNO_3.

Synchronized development and maturation of somatic embryos

Table 4. Effects of sucrose and glucose on the development of globular embryos of *O. javanica* (Bl.) DC. in suspension culture with MS medium under dim light for 3 weeks.[a]

Carbohydrate (%)	Fresh weight (mg)	No. of globular embryos	No. of mature embryos	Root length [b]	Shoot growth[c]
Sucrose					
0	80.3	86.9	24.1	-	-
3	1150.7	550.3	57.4	++	++
6	1050.4	480.3	63.4	+++	+
9	220.3	98.4	19.3	+	+
12	180.9	72.1	20.7	-	-
Glucose					
3	52.7	100.3	2.0	-	-
6	53.3	90.3	2.5	-	-
9	42.6	50.1	5.5	-	-
12	41.1	65.8	4.7	-	-

[a]Data collected from 3 replicates of 100 ml flask. [b] -: no rooting, +: below 2 mm, ++: 2 - 4 mm and +++ : 4 mm or longer. [c] -: no shooting, +: poor and ++: medium.

Table 5. Effects of ABA on the growth and development of globular embryos of *O. javanica* (BL.) DC. cultured in MS suspension medium for 3 weeks.[a]

ABA (μM)	Globular embryos [b]	Mature embryos [c]	Root length [d]	Shoot growth [e]	Hypocotyl length [f]
0	++++	+++	++++	++	++++
1	+++	+++	+++	+++	+++
3	+++	+++	++	++	+++
6	++	++++	+	++	++
10	+	++++	+	++	+

[a] 100mg fresh weight was inoculated in MS liquid media.
[b, c] +: a few, ++: medium, +++: many and ++++: very many.
[d, f] +: very short, ++: short, +++: medium and ++++: long.
[e] +: none, ++: poor, +++: medium and ++++: good.

Embryo development was greatly influenced by the sucrose concentration in the media (Table 4). The fresh weight and the number of developing embryos were the best in the media containing 3% or 6% sucrose. Glucose did not promote embryo development at any concentration. Kim and Lee (1995) reported that 6% sucrose enhanced maturity of somatic embryos in *O. stolonifera*. Ammirato and Steward (1971) reported that increased sucrose concentration prevented precocious embryo germination in a number of species including *Daucus carota*. In *O. javanica* (Bl.) DC., 3 - 6% sucrose was good for maturation of embryos, but sucrose at higher concentration than 6% prohibited the growth of somatic embryos.

Globular embryos developed into the abnormal embryos having long roots, elongated hypocotyl and deformed shoot on basal medium without ABA. Addition of ABA to the culture medium, however, resulted in uniform and synchronized development of normal embryos, repressing the formation of secondary embryos and embryogenic cell clumps (Table 5)(Figure 2A). As the concentration of ABA increased, roots and hypocotyls became shorter (Figure 2B). When 1.0 μM ABA was added to the media, embryo development became uniform and synchronized but shoot development was not satisfactory. It was reported that the appearance of abnormal embryos with extremely elongated roots was suppressed by application of ABA at a physiological concentration during embryogenesis. (Kamada and Harada, 1981), (Ammirato, 1974, 1977). When ABA was added at 10 μM to the liquid media, the embryos became normal and produced less

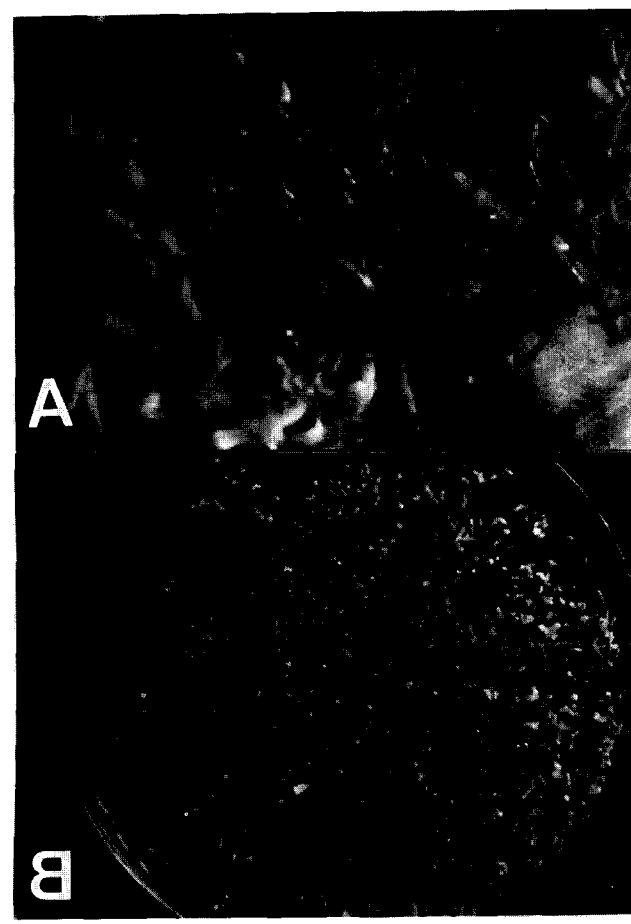

Figure 2. Development of somatic embryos of *O. javanica* (Bl.) DC. on the media containing various concentrations of ABA. A: Somatic embryos with elongated hypocotyls developed on the medium free of ABA. B: Normal embryos developed in the medium containing 10 μM ABA.

secondary embryos. Elongation of hypocotyl and root was also suppressed. Therefore, to eliminate abnormalities observed in the somatic embryos, exogenous application of ABA was

Table 6. Effects of BA and NAA on the growth and development of globular embryos of *O. javanica* (Bl.) DC. on MS solid media during 4 weeks under continuos light or dark condition.

Illumination	BA (mg/L)	NAA (mg/L)	Fresh weight (mg)	No. of embryos ( ) 0.5 cm)	No. of plantlets (0.5 - 1.0 cm)	No. of plantlets (1.0 cm ())
Light	0.0	0.0	830±380 [z]	7.0±2.2 [y]	8.6±3.7 [y]	3.6±1.7 [y]
		0.1	2020±770	8.6±3.6	9.0±6.3	3.0±1.1
		0.5	220± 10	7.0±3.3	7.0±2.3	-
		1.0	150± 30	5.8±3.3	8.0±2.2	-
		2.0	150± 30	9.4±2.9	8.2±3.7	-
	0.1	0.0	950± 30	8.0±3.1	8.0±2.1	5.5±1.2
		0.1	2080±640	5.5±2.7	4.0±3.9	8.2±3.6
		0.5	440±140	8.6±1.3	12.4±1.7	5.2±1.6
		1.0	162± 30	7.0±3.1	7.0±3.1	-
		2.0	140± 30	9.0±2.5	6.0±2.2	-
Dark	0.0	0.0	595± 49	17.0±4.2	4.5±3.1	6.8±3.2
		0.1	783±116	12.5±6.5	10.0±4.7	8.0±5.1
		0.5	675± 21	8.2±2.4	9.5±1.3	6.3±2.2
		1.0	383± 26	10.5±2.1	7.6±2.2	6.3±1.0
		2.0	384± 46	-	-	-
	0.1	0.0	398±49	17.7±1.7	6.8±1.0	7.0±1.4
		0.1	413±69	18.8±4.6	6.3±1.0	4.5±2.4
		0.5	363±64	-	-	-
		1.0	375±93	-	6.0±1.8	1.3±3.5
		2.0	308±57	-	9.8±1.3	3.8±0.5

[z]Mean fresh weight ± S.D and [y] Mean number ± S.D. Data collected from 3 replicates of 100 mL flask.

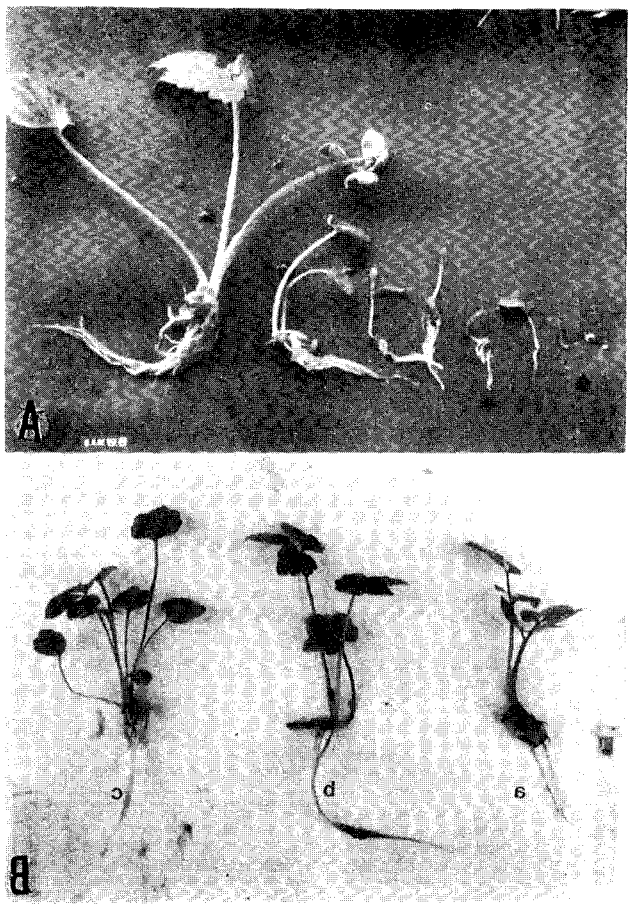

Figure 3. Growth and development of somatic embryos of *O. javanica* (Bl.) DC. A: Development of a globular embryo to a plantlet. B: Comparison of plantlets from cutting (a), from shoot tip culture (b), and from somatic embryo (c)

found to be effective.

Conversion of the somatic embryos into plantlets

Development of embryos and plantlets was the best in the media containing NAA at 0.1 mg/L and BA at 0.1 mg/L under light condition (Table 6). However, the number and fresh weight of plantlets decreased on the media containing NAA above 0.1 mg/L. Development of plantlets was not observed on the media containing 2 mg/L NAA. The number of embryos produced under light control was similar to that under dark condition but the size of embryos was much larger under light condition. The globular embryos and embryogenic cell clumps transferred to solid media developed into mature embryos. The mature embryos germinated into plantlets on the same media. By this way, 50 - 100 plantlets per 100 mL flask were obtained. The plantlets were transplanted to perlite or directly to the water-logged soil in the pot with or without acclimatization. The plantlets grew well into established plants.

Plantlets from somatic embryos were similar to the seedlings. Cotyledons and primary leaves were entire and oval in shape. As the plantlets grew, the first true leaves became trifoliate and the later ones multifoliate. Meanwhile the initial leaves of the plantlets obtained through shoot tip culture or cuttings were trifoliate or multi-foliate. The plantlets obtained from the somatic embryos grew more vigorously than those from cutting.

Some plantlets raised from somatic embryos had 3-6 cotyledons, duing to hormonal or nutritional unbalance but not duing to genetic variation. Subculture on basal medium reduced the frequency of multi cotyledonary plantlets, which became normal plants after transfer to the fields (Figure 3A, B).

摘 要

본 硏究는 미나리의 胚發生 캘러스를 培養하여 正常的이고 均一한 形態의 體細胞 胚를 大量 生産할 수 있는 條件을 究明하고, 生産된 體細胞胚를 種苗로 利用하기 위한 基礎

硏究로 實施하였다. 胚發生 캘러스를 生長調節劑, 糖, 窒素源 등을 濃度別로 添加한 MS培地에 懸濁培養한 結果는 아래와 같다.

胚發生 캘러스를 生長調節劑가 添加되지 않은 液體培地에 懸濁培養하면 胚發生 細胞塊 및 體細胞 胚가 旺盛하게 增殖되었다. 球形段階의 胚를 IBA와 NAA를 添加한 培地에 懸濁培養하면 胚發生 細胞塊의 增殖과 함께 배의 發達도 進行됐으나, 2,4-D가 添加된 培地에서 培養하면 胚發生 細胞塊 및 細胞만 增殖하고 胚의 發達은 進行되지 않았다. 窒素源의 種類에 따른 胚發生 細胞塊의 生長 發育狀態에서 KNO_3와 NH_4NO_3가 各各 20 mM 添加되었을 때 가장 좋았다. 幼芽 發達에는 NH_4NO_3가, 幼根發達에는 KNO_3가 效果的이었다. 球形段階의 배를 sucrose가 3 - 6 % 添加된 培地에 培養하면 發育이 좋았다. 球形段階의 胚를 ABA가 添加되지 않은 培地에 培養하면 下胚軸의 異常伸長 및 多胚現像이 일어나지만 ABA가 10 μM 添加된 培地에 培養하면 下胚軸의 길이가 短縮되고 二次胚 發生도 顯著히 減少하였다. 固體培地에 胚發生캘러스를 繼續 培養하여 多量의 球形胚를 얻을 수 있고 이들을 生長調節劑가 添加되지 않은 培地에서 培養하면 種子 由來 植物體와 類似한 幼植物을 얻을 수 있었다.

REFERENCES

Ammirato PV (1969) Morphological responses of cultured cells and tissues. PhD dissertation, Cornell University, NewYork

Ammirato PV (1974) The effects of abscisic acid on the development of somatic embryos from cells of caraway (*Carium carvi* L.). Bot Gaz **135**: 328-337

Ammirato PV (1977) Hormonal control of somatic embryo development from cultured cells of caraway; Interactions of abscisic acid, zeatin, and gibberellic acid. Plant Physiol **59**: 570-586

Ammirato PV, Steward FC (1971) Some effects of environment on the development of embryos from cultured free cells. Bot Gaz **132**: 149-158

Kamada F, Harada H (1981) Changes in the endogenous level and effects of abscisic acid during somatic embryogenesis of *Daucus carota* L. Plant & Cell Physiol **22**: 1423-1429

Kim BW (1986) Seed development and germination characteristics of *Oenanthe stolonifera* DC. PhD dissertation, Seoul National University

Kim HS, Lee BY(1995) *In vitro* production system of somatic embryos in *Oenanthe stolonifera* DC. J Kor Soc Hort Sci **36**: 38-45

Kim YH, Kim HI, Chung TY, Harn C (1988) Induction of somatic embryos and germination of encapsulated embryos in celery. Kor J Plant Tiss Cult **13**: 129-136

Kitto SL, Janick J (1985) Production of synthetic seeds by encapsulating asexual embryos of carrot. J Amer Soc Hort Sci **110**: 277-282

Kitto SL, Janick J (1985) Hardening treatments increase survival of synthetically-coated asexual embryos of carrot. J Amer Soc Hort Sci **110**: 283-286

Koh GC, Ahn CS (1995a) Production and Developmental pattern of embryogenic callus in *Oenanthe javanica* (Bl.) DC. Kor J Plant Tiss Cult **22**: 283-289

Koh GC, Ahn CS (1995b) Anatomical observation of somatic embryogenesis in *Oenanthe javanica* (Bl.) DC. Kor J Plant Tiss Cult **22**: 323-327

Redenbaugh K, Viss P, Slade D, Fuji A (1987) Artificial seeds, *In* Plant tissue and Cell Culture, Green, C E, eds, Liss, A R, NewYork, pp 483-493

Yang SY (1986) A Study on the strain classification of Korean water dropwort (*Oenanthe javanica* DC.). PhD Dissertation, Chonnam National University

(1997년 1월 3일 접수)