Korean J. Medicinal Crop Sci. 12(2) : 118 - 122 (2004)

Bioceramic Effects to Enhance Secondary Metabolites Production in Tissue Culture of Some Medicinal Plants

Yu Jeong Kim*, Baik Hwang*, and Jun Cheul Ahn**†

*Department of Biology, Chonnam National University, Gwangju 500-757, Korea.
**Department of Biology, Seonam University, Namwon 590-170, Korea.

ABSTRACT : We have investigated that a couple of soft ferrite ceramic powders having a spinal structure have shown the effect on growth and secondary metabolites production of some medicinal plants cultured *in vitro*. The addition of the ceramic powders as bare state to culture medium has stimulated the growth of *Achyranthes japonica* callus and plantlet, adventitious root of *Hyoscyamus niger* and *Platycodon grandiflorum* hairy root about 65, 75, 150 and 50%, respectively. Whereas *Centella asiatica* callus and plantlet, *Scopolia parviflora* hairy root, and *Hyoscyamus albus* adventitious root were not affected markedly. Moreover, the ceramic powder has enhanced the growth of *H. niger* adventitious roots even under conditions of irradiating alone without any direct contact between ceramic powder and media. Based on growth stimulation effect, the ceramic powders have enhanced the gross production of tropane alkaloid in *H. niger* adventitious root, and polyacetylene in *P. grandiflorum* hairy root about 35 and 30%, respectively.

Key words : callus, adventitious root, hairy root, plantlet, secondary metabolite

INTRODUCTION

The far infrared radiating ceramics, made by mixing 20 kinds of non-carbonization ceramics, including silundum, silica, and nitrogenous compounds, with clay or metal and plasticizing the mixture at 1,600~1,800℃, absorb electromagnetic waves of all ranges from short wavelength to long wavelength and turn such absorbed energy into heat energy of far infrared. The far infrared rays from this ceramic have the wavelength of 5.6 to 15 micron and are weak. However, if they are irradiated to animals or plants, they are resonating at the same wavelength range as that to be absorbed by cellular proteins or other macromolecular compounds to activate living cells, resulting in improvement of metabolism or promotion of growth. Therefore, their freshness is maintained for longer periods. Further, there is a report that such irradiated animals or plants have the improved resistance against pathogenic microorganisms (Iwade *et al.*, 1989; Miyake, 1989). Accordingly, such ceramics have been used to dry grains or produce soft water and to treat the bruise for medical purpose (Uchida *et al.*, 1987). The ceramics are used to germinate plant seeds (hot pepper, lettuce, tomato, or radish) or grow the rice plant (Chung *et al.*, 1992). Moreover, a study reported that the silver-treated ceramics are effective in cultivating the radish or bean sprouts (Kim *et al.*, 1997).

On the other hand, plant tissue culture has been considered to be an alternative to natural plant extraction, to produce valuable biochemicals and to offer simplicity and potential economical benefits. Recently, in order to improve the yield of product in culture system, various techniques have been tried including manipulation of culture conditions, alteration

† Corresponding author : (Phone) +82-63-620-0256, (E-mail) jcahn@tiger.seonam.ac.kr
Received March 20, 2004 / Accepted April1 19, 2004.

of cell metabolism and elicitor treatment (Sandra *et al.*, 2000).

We discuss here on the effect of two kinds of soft ferrite ceramic powders on growth of callus, adventitious roots, hairy roots, and plantlets of some medicinal plants, and effect on productivity of secondary metabolite.

MATERIALS AND METHODS

Ceramic powders and plant materials

Ceramic powders: Both A (Fe_2O_3, 42.46%; ZnO, 9.97%; MgO, 38.9%; MnO_2, 7.6%; Dy_2O_3, 0.73%; RuO_2, 0.23%) and B (Fe_2O_3, 61.4%; ZnO, 26.1%; MnO_2, 5.6%; Dy_2O_3, 0.62%; RuO_2, 2.3%) ceramic powders containing Fe_2O_3 as major ingredients were used, and the synthesis was in accordance with the method by Kim *et al.* (1997). After the material measured in a weight rate, these crushed and mixed. This mixture was mixed with distilled water with a volume ratio of 1:1.5 and then crushed for 2 hrs by wet mixing the mixture with respect to the ball with a weight ratio of 2.5:2.0. The compound crushed sample was removed a moisture by dring for 48 hrs with the constant temperature dryer adjusting 110℃. The dried block was crushed and filtered with a sieve of 70 mesh and then the powder was plastic at 1250℃ for 3 hrs in crucible. These again was crushed during 2 hrs by using a Wet Ball Milling method. The crushed sample dried enough in a constant temperature dryer at 110℃ and prepared it. We sieved this prepared sample and used 38~58 ㎛ size powder as a sample for this study.

Plant materials: callus of *Achyranthes japonica, Centella asiatica* and *Ginkgo biloba*, adventitious root of *Hyoscyamus niger* and *Hyoscyamus albus*, hairy root of *Scopolia parviflora, Bupleurum falcatum* and *Platycodon grandiflorum* and young plantlet of *A. japonica, C. asiatica* and *S. parviflora* established *in vitro* were used. The plant materials were obtained from the laboratory of plant physiology, Department of Biology, Chonnam National University, Gwangju.

Culture conditions

Culture conditions of each sample are summarized in Table 1. For all the experiments, segments of

Table 1. Culture condition of callus, adventitious root, hairy root and young plantlet used in this experiment.

	Materials	Culture conditions					
		Basal Media	Sucore (%)	pH	Inoculation weight	Culture Period	rpm
Callus	*Achyranthes japonica*	SHA†	3	5.8	0.5 g	3 weeks	100
	Centella asiatica	MSB‡	3	5.8	0.5 g	〃	〃
	Ginkgo biloba	MSC§	3	5.8	0.5 g	〃	〃
Adventitious root	*Hyoscyamus niger*	MS	3	5.8	3 root tips (Ca. 100 ㎎)	3 weeks	100
	Hyoscyamus albus	WP	3	5.7	〃	〃	〃
Hairy root	*Scopolia parviflora*	1/2B5¶	5	5.6	3 root tips (Ca. 100 ㎎)	3 weeks	100
	Bupleurum falcatum	2White‡	3	4.8	〃	8 weeks	〃
	Platycodon grandiflorum	1/2B5	3	5.6	〃	4 weeks	〃
Plantlet	*Achyranthes japonica*	MS	3	5.8	1 axillary bud	4 weeks	–
	Centella asiatica	1/2B5	3	5.6	meristem (Ca. 300 ㎎)	〃	–
	Scopolia parviflora	MS	3	5.8	1 axillary bud	〃	–

†SHA, SH medium containing 2,4-D 1 ㎎/ℓ ; ‡MSB, MS medium containing NAA 1 ㎎/ℓ ; §MSC, MS medium containing NAA 1 ㎎/ℓ and 0.1 ㎎/ℓ kinetin; ¶1/2 B5, half strength of macro elements medium of B5; ‡2White, double strength of macro elements medium of White.

callus, root, and plantlet were cultured in 30 ml of medium/100 ml Erlenmeyer flask. To examine the effect of ceramics without direct contact between ceramic powders and media ingredients, *H. niger* adventitious root was cultured under the condition in that ceramic powders are packaged only in translucent glass tube immersed in 100 ml Erlenmeyer flask (Fig. 1). Three root tips per a flask were inoculated into the flask containing 30 ml of free-hormone MS liquid medium with 3% sucrose and cultured on a rotary shaker (100 rpm) at 25℃ in the dark conditions for three weeks.

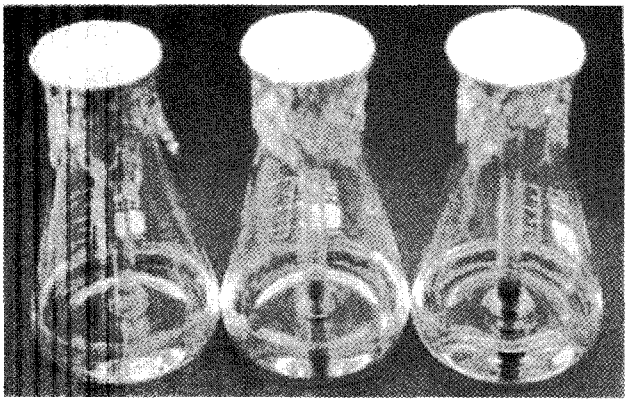

Fig. 1. Erlenmeyer flask for survey of ceramic powders -effect of the growth of plant under non-contact condition.

Polyacetylene and tropane alkaloid analysis

In polyacetylene analysis of P. grandiflorum, 20 mg of each lyophilized sample was extracted with 2 ml MeOH for 15 hrs at 4℃ and HPLC analysis was conducted by Ishimaru *et al.* (1993). In tropane alkaloid analysis of *H. niger*, 50 mg of each sample was extracted with 5 ml $CHCl_3$-MeOH-NH_4OH (15:5:1) mixture using sonication (10 min.). After purification the alkaloid extracts were dissolved in MeOH and analyzed by HPLC (Waters Co.), column ODS (4.6 mm ID x 25 cm), MeCN-10 mM SDS (2:3, pH 3.3), flow rate 1.1 ml/min., UV at 215 nm as described by Shimomura *et al.* (1991).

RESULTS AND DISCUSSION

The effects of ceramic powders on growth are shown in Table 2. Treatment with ceramic B at 0.1% in *A. japonica* cell suspension was stimulated about 60% in growth. In *C. asiatica* and *G. biloba* cell suspension, growth rates were enhanced 15% with ceramic B at 0.05% and 17% with ceramic A at 0.05%, respectively. In root culture, growth of *H. niger* adventitious root (Fig. 2) and *P. grandiflorum* hairy root cultured with ceramic B at 0.05% grew faster by up to about 140% and 50% compared with the control, respectively. However, growth rate of *S.*

Table 2. Effect of soft ferrite ceramic powders (A, B) on the growth in callus, adventitious root, hairy root and young plantlet culture of some medicinal plants†.

Materials		Ceramic A conc. (%)				Ceramic B conc. (%)			
		0	0.01	0.05	0.1	0	0.01	0.05	0.1
Callus	*Achyranthes japonica*	–*	–	–	–	100	–	125	163
	Centella asiatica	100	98	110	–	100	100	115	–
	Ginkgo biloba	100	108	117	–	100	108	83	–
Adventitious root	*Hyoscyamus niger*	100	100	125	–	100	192	242	–
	Hyoscyamus niger (N.C)§	–	–	–	–	100	125	154	135
	Hyoscyamus albus	100	83	120	–	100	130	75	–
Hairy root	*Scopolia parviflora*	–	–	–	–	100	104	104	–
	Bupleurum falcatum	–	–	–	–	100	102	126	–
	Platycodon grandiflorum	–	–	–	–	100	120	150	–
Plantlet	*Achyranthes japonica*	100	–	163	–	100	–	175	–
	Centela asiatica	100	100	105	–	100	94	99	–
	Scopolia parviflora	100	–	100	–	100	–	127	–

†Effect is represented with mean % of three replicates (compared with control, 100%). * –, not examined. §(N.C), non-contact condition.

parviflora hairy root was not affected and *H. albus* adventitious root grew a slight higher at ceramic A of 0.05% and ceramic B of 0.01% treatment. In plantlet culture, growth rate of *A. japonica* plantlet at ceramic A and B, 0.05% treatment increased by about 63% and 75%, respectively (Fig. 3). Whereas, there was not difference in growth rates of plantlet of *C. asiatica* at all the conditions tested and growth rate of *S. parviflora* plantlet at ceramic B of 0.05% only slightly increased. In addition, even under culture conditions without direct contact between the ceramic powders and media, growth rate decreased a little than that of direct addition but stimulation effect of the powders was also confirmed in *H. niger* adventitious root. On the basis of these results, the effects of ceramics on productivity of some secondary metabolites were examined. In major tropane alkaloid production of *H. niger* and polyacetylene production of *P. grandflorum*

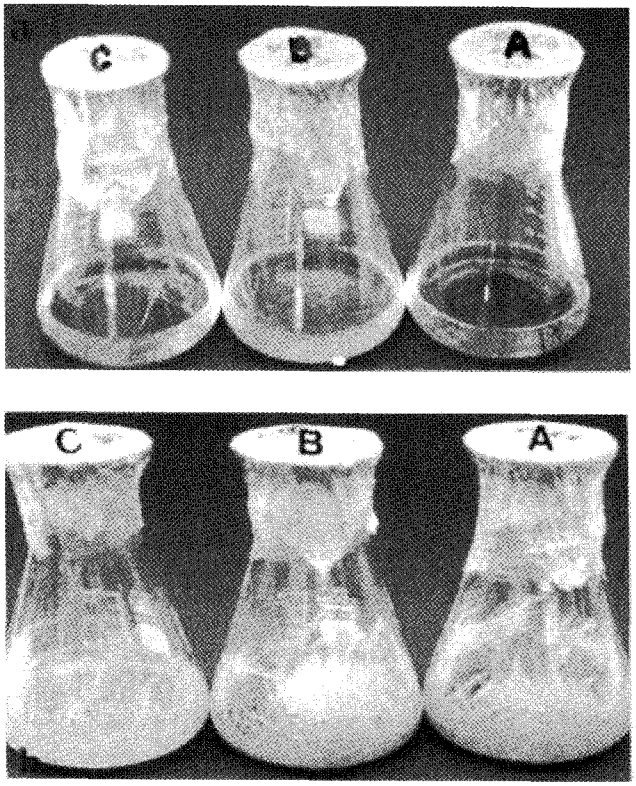

Fig. 2. Effect of ceramic powders on the growth of *Hyoscyamus niger* adventitious root after 1 week (upper) and 3 weeks (lower) culture in MS liquid medium. C, control; A, 0.05% ceramic powder A; B, 0.05% ceramic powder B.

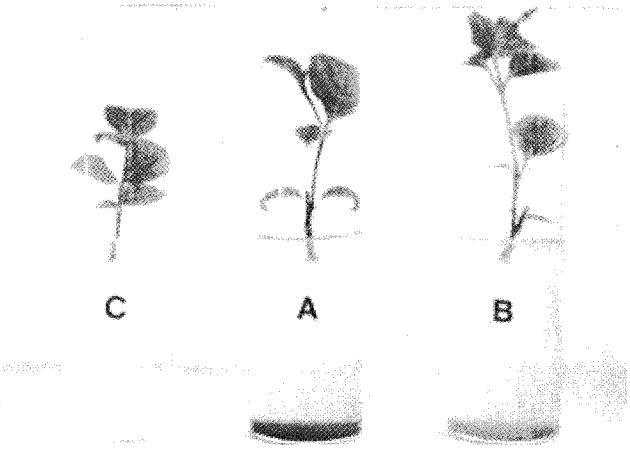

Fig. 3. Effect of the ceramic powders on the growth of *Achyranthes japonica* plantlets after 4 weeks culture in MS solid medium. C, control; A, 0.05% ceramic powder A; B, 0.05% ceramic powder B.

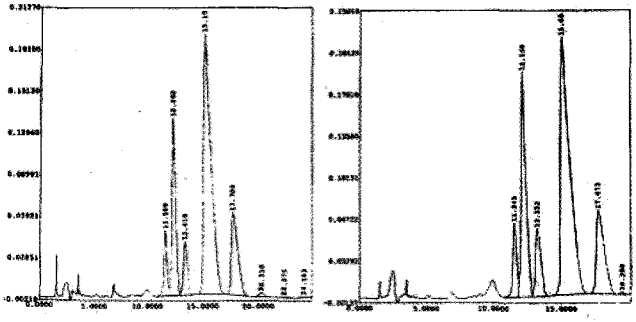

Fig. 4A. HPLC chromatogram of control (left) and 0.05% ceramic B treatment (right) in *Hyoscyamus niger* adventitious root culture.

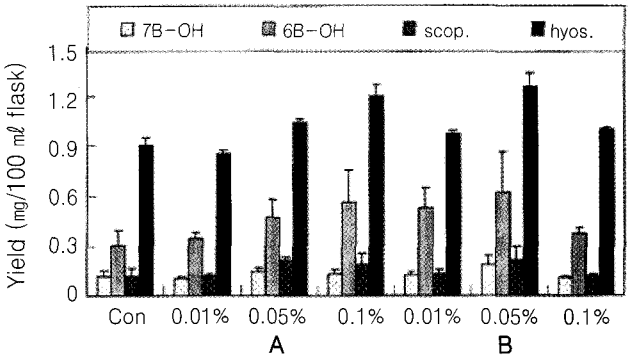

Fig. 4B. Effect of the ceramic powders on growth and tropane alkaloids production of *Hyoscyamus niger* adventitious root after 3 weeks culture in MS liquid medium. Values in brackets show the dry wt; 7B-OH, 7β-hydroxyhyoscyamine; 6B-OH, 6β-hydroxyhyoscyamine; scop, scopolamine; hyos, hyoscyamine. CON; control.

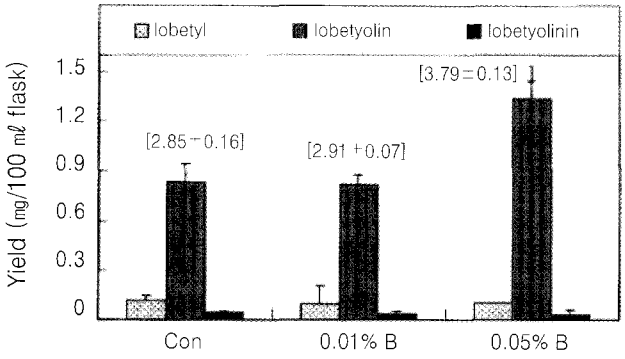

Fig. 5. Growth and polyacetylene production *Platycodon grandiflorum* hairy root D 10 cultured in 1/2 B5 liquid medium for 4 weeks at 25℃ in the dark. Values (g/100 mℓ flask) in brackets show the fresh weight. Bars represent standard errors. CON; control.

with ceramic B of 0.05% treatment, yields increased about 35 and 30%, respectively, and there was no difference in pattern of tropane alkaloid between ceramic treatment and control in *H. niger* adventitious root (Fig. 4A, B; Fig. 5).

Two soft ferrite ceramic powders on growth of callus, adventitious root, hairy root, and plantlet cultured in vitro were found to be effective, but degrees of the effect were represented differently, depending on species or culture type tested. Similar results were obtained in study using a ceramic powder with some vegetables (Chung *et al.*, 1992; Kim *et al.*, 1997). Chung *et al.* (1992) reported that treatment with 0.5 ㎎/ℓ of another ceramic (BL 700) resulted in desirable effects on germination of lettuce and tomato seeds and growth of epicotyls and hypocotyls and such effects were resulted from thermal action by far infrared rays from the ceramic. Also, Chung *et al.* (1992) applied the same ceramic in powder, ball, and pad forms to cucumber under water culture and reported that it resulted in increased growth and harvest. Further, Lee *et al.* (1997) applied Al_2O_3 ceramic powders to the rice plant seedlings. These results indicated that far infrared rays were well absorbed into their cells to cause the resonance action with cellular water molecules, resulting in promotion of metabolism and growth.

On the other hand, from the facts that growth rate of *A. japonica* was stimulated in both callus and plantlet and that of *C. asiatica* which did not show the effect in both, it was inferred that the effect may be concerned with cell level regardless of differentiation or de-differentiation of plants. Also, the results presented above showed that the effect of ceramic A on growth rate was higher than ceramic B, suggesting that there may be some correlation between the effect on plants and the difference of ceramic ingredients or making processing of ceramic. In particular, because there was the effect on growth without direct contact between ceramic powder and media, only if ceramic powders were not role of an adsorbent of gas such as ethylene, it was inferred that the effect depends on physical principle such as far-infrared or magnetism.

ACKNOWLEDGEMENT

This work was supported by a grant from Biogreen 21 program, Rural Development Administration, Republic of Korea.

LITERATURE CITED

Chung SJ, Lee BS, Lee JP (1992) Germination responces of lettuce and tomato seeds as affected by the treatment of bioceramec powder. Agriculture Forestry and Fishery 37:59-66.

Chung SJ, Lee BS, Lee JP (1992) Effects of bioceramics in root-zone on the growth and fruit yield of hydroponically grown cucumber. Agriculture Forestry and Fishery 37:67-76.

Ishimaru K, Arakawa H, Sacoshima S, Yamaguchi Y (1993) Effects of basal media on growth and polyacetylene production of *Lobelia inflata* hairy roots. Plant Tiss. Cult. Lett. 10:191-193.

Iwade K, Kitamura N, Nakamura K (1989) Preparation of far-infrared radiator. New Ceramic 1:81-88.

Kim KS, Sun HS, Bae KW, Park CY (1997) Disinfecting effect and growth enhancement of silver coated ceramic powder in vegetables. Korean J. Biotecnol. Bioeng. 12:35-39.

Lee CW, Han CS, Son SY (1997) Effect of ceramics on growth of rice seedings for machine transplanting. Korean J. Crop Sci. 42:141-145.

Miyake J (1989) Far infrared radiation and a living body. New Ceramic 1:101-104.

Sandra IP, Tatiana CS, Ana MG (2000) The influence of different biotic and abiotic elicitors on the production and profile of tropane alkaloids in hairy root cultures of *Brugmansia candida*. Enzyme and Microbial Technology 26:252-258.

Shimomura K, Sauerwein M, Ishimaru K (1991) Tropane alkaloids in the adventitious and hairy root cultures of Solanaceous plants. Phytochemistry 30:2275-2278.

Uchida AS, Nade S, McCartney E, Ching W (1987) Growth of bone marrow cells on porous ceramics *in vitro*. J. Biomed. Matter. Res. 21:1-10.