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Mass production of Siberian Ginseng (*Eleutherococcus senticosus*) somatic embryos by cell culturing

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SUMMARY

Somatic embryogenic cells of Siberian ginseng (*Eleutherococcus senticosus* or *Acanthopanax senticosus*) were cultured in MS liquid medium by subculture at 2 week intervals. The embryogenic cells were filtered through a 250 μm sieve and the passed cells could proliferate with maintenance of somatic embryos. The embryogenic cell clusters entrapped on the sieve were transferred to an MS medium without growth regulators and were cultured under fluorescent light in the half shade. The cell clumps developed to somatic embryos of uniform sizes of torpedo stage after 4~5 weeks of culture. The somatic embryos in a torpedo stage were found to gain in fresh weight about 20 times greater after culturing in the bio-reactors. The somatic embryos in a torpedo stage were transferred to 510 L air-left type bioreactors. The culturing for 10~15 days led the somatic embryos to the development of seedlings which could be utilized as materials for health foods or providing useful components.

Key words: Bioreactor, Cell culturing, Mass production, Siberian ginseng

INTRODUCTION

Siberian ginseng (*Eleutherococcus senticosus or Acanthopanax senticosus*), which is known as an efficacious medicinal herb all over the world, grows naturally in the Far Eastern Asia (Korea, Siberia, China and Hokkaido). Since the first report of disclosing the value of Siberian ginseng as a medicine, researchers in many countries, including Russia, have made efforts to reveal its medicinal components. As a result, acanthoic acid, which is evaluated as being five times more potent in anti-inflammatory activity than that of aspirin, β-sitosterol, eleutheroside A~G, and stigmasterol extracted from Siberian ginseng. Eleutheroside E, which shows various physiologically active effects, is contained in the Siberian ginseng growing to Korea. This compound contained at an amount 1.7~5.5 times greater than that of Acanthopanax spp. such as Acanthopanax chiisanesis, *Acanthopanax seoulense, Acanthopanax sieboldianum* and *Acanthopanax koreanum* (Park, 1997).

However, Siberian ginseng is difficult for general farm households to seed because of its stringent weather conditions. In addition, the stem cutting of the plant is not effective for propagation. Also, because medicinal materials are taken from the velamen and bark of Siberian ginseng, it takes a long period of time for this shrub to be cultivated to useful extent. In addition, the worst thing is, once the velamen and the bark are taken off, the plant no more maintains its existence. That is, this plant cannot afford reproductive provisions of the medicinal materials, but gives only one chance for getting the medicinal materials.

There are reports regarding the generation of adventitious buds through tissue culture or the regeneration of plants through somatic embryogenesis (Gui *et al*., 1991; Choi and Soh, 1993). Achieved

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only in small scales on agar media, the techniques disclosed in the reports are different from those of the present paper in which embryogenic cell strains are established in a liquid medium and used to produce plantlets in a large quantity. Up until now, there has been reported no research on the system for commercializing Siberian ginseng as healthy foods by mass producing Siberian ginseng through tissue culture.

MATERIAL AND METHODS

Embryogenic cells of Siberian ginseng were cultured in a 1 L Erlenmeyer flask containing 300 ml of MS (Murashige and Skoog, 1962) liquid medium supplemented with 3% sucrose without growth regulators. The pH of the medium was abjected to 5.8, and autoclaved at 121°C at 1.2 atm for 15 min. All cultures were maintained in a shaking incubator at 25°C, dark condition while shaking at 100 rpm. At 2 weeks after culturing, about one tenth of the total wet weight of the cell clusters passed freely through a 250 μm stainless steel sieve. These small sized clusters could proliferate with maintenance of somatic embryos. The cell clusters entrapped on the sieve, amounting to nine tenths of the total wet weight, were transferred in a 5 L vessel containing 2 L of an MS liquid medium without growth regulators and cultured under fluorescent light in the half shade.

In order to classify the cell clusters and globular embryos, the cells are settled for 1-2 min. For globular embryos settled in a flask bottom are scanted to another vessel. The somatic embryos were transferred to air-lift type bioreactors containing MS basal medium supplemented with 2% sucrose. The bioreactors used were designed to allow germ-free air to be introduced to their lower parts with the aid of compressors or air pumps for aquaria. The bioreactor equipped to their lower parts with air compressor, air filter (Midisart 2000, Sartorius) employed filteration with .0.2 μm.

To determine whether the plantlets cultured in a bio-reactor were contaminated or not, they were washed with a sterilized water and then cultured at 25±1°C for 3 to 4 days on petridishes at an amount of 4~5 g per dish. The plantlets, if free of germs, remained intact without denaturation when being stored in a refrigerator.

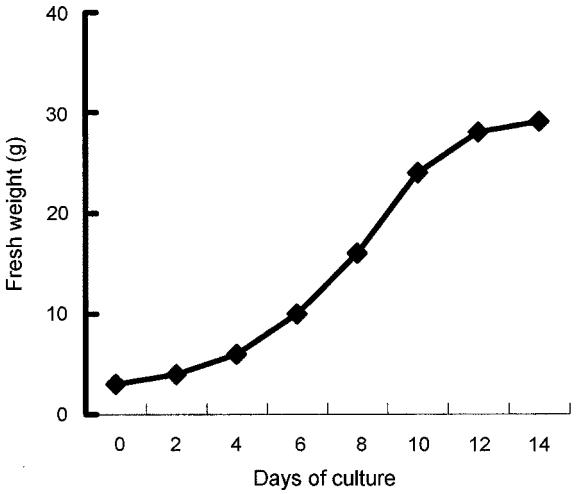

Fig. 1. Growth curve of embryogenic cells of Siberian ginseng cultured in a 5 L vessel containing 2 L of an MS liquid medium without growth regulators.

RESULTS

Embryogenic cells of Siberian ginseng were suspension cultured in an MS liquid medium. The embryogenic cells grows about 10 times greater after 2 weeks in cultures (Fig. 1). About one tenth of the cell clusters passed freely through a 250 μm stainless steel sieve(Fig. 2A). When cultured in fresh medium, these cells and small sized clusters could proliferate and developed to somatic embryos with maintenance of identical embryogenesis. Therefore, this process makes the embryogenic cells permanently maintained and proliferated.

On the other hand, the cell clusters entrapped on the sieve, amounting to nine tenths of the total fresh weight, were transferred in MS medium without growth regulators and cultured under fluorescent light in the half shade. It took about 2 weeks for most of the cell clusters to be developed to globular embryos. In order to classify into homogeneous sizes, the subculture was allowed to stand for 1~2 min for globular embryos to settle down, after which the medium was scanted to another vessel. Light cell clusters were transferred to the vessel, together with the medium, while most of the remainders were the globular embryos, heavier than the cell clusters. Like this, the synchronous subculture taking advantage of

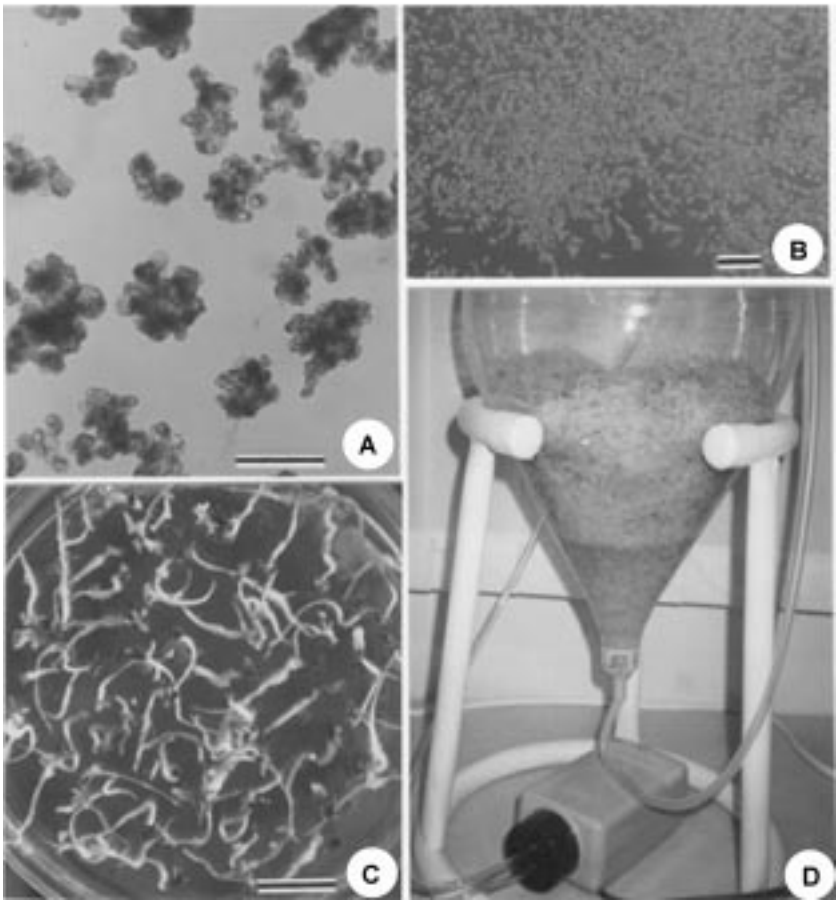

Fig. 2. Induction of somatic embryos and mass production of plantlets of Siberian ginseng through cell culture. A. Cells and cell clusters cultured in MS medium without growth regulators (bar=0.5 mm). B. Somatic embryos induced from embryogenic cells (bar=3 mm). C. Plantlets regenerated form somatic embryos (bar=10 mm). D. Mass production of plantlets by culturing in 10 L air-lift type bioreactor.

gravity allowed relatively homogeneous sizes of somatic embryos to be collected in a vessel during the development of somatic embryos. Globular embryos of Siberian ginseng were developed to embryos of uniform sizes of torpedo by suspension culturing with subculturing every 2 or 3 weeks. (Fig. 2B).

Uniform sizes of a cotyledon shape stage (about 3-5 mm) were developed by suspension culturing in MS medium without growth regulators with subculturing every 2 or 3 weeks. The somatic embryos in a torpedo- or cotyledon-stage were allowed to grow in MS medium (2% sugar) in a 10 L air-lift type bioreactor(Fig. 2D).

The somatic embryos in a torpedo shape stage were found to gain in fresh weight about 20 times greater after culturing in the bio-reactors for about 14 days than before the culturing while 10 times greater fresh weights were measured for the plantlets developed from the somatic embryos in a cotyledon stage than the embryos (Fig. 3). The

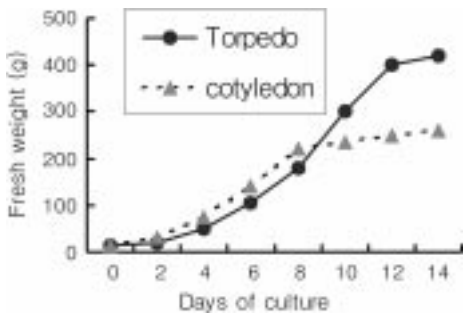

Fig. 3. Growth curves of a torpedo shape stage (●) and a cotyledon shape stage (▲) in cultured on 10 L air lift type bioreactors.

culturing for a period of about 10~15 days led the somatic embryos to the development of plantlets (Fig. 2C).

DISCUSSION

In the case of Siberian ginseng, there have been found almost no cases in which the plantlets were regenerated through cell culturing. Therefore, the establishment of stable and high producing cultures condition is necessary for successful plantlet production. The present paper described in detail methods that provide plantlets of Siberian ginseng in a highly efficient manner. Somatic embryos have been reported to be morphologically different compared to zygotic embryos. Abnormal morphology such as poor cotyledon development, long roots or callused embryos have been frequently observed (Pence *et al.,* 1980; Kim and Soh, 1996; Choi *et al.,* 1997). Therefore, the rate of plantlet conversion from somatic embryos was low compared to zygotic embryos. These are the main barriers for commercial application of mass propagation (Green and Phillips, 1975). However, most somatic embryos of Siberian ginseng had a normal morphology and showed a low frequency of abnormal embryos. This result indicates that somatic embryogenesis of Siberian ginseng might be a promising system of in vitro plantlet propagation.

In the case of ginsengs (*Panax ginseng*), mass culturing of their cells was successfully accomplished in culture vessels, enabling the extraction of saponin therefrom (Furuya *et al.,* 1983). The cultured cells are commercially sold as a healthy food after being dried and powdered in their entirety without processing. In addition, on the pharmaceutical studies *in vitro* the cultured ginseng was more effective than cfield grown ginseng (Hibino and Ushiyama, 1996). The multiple adventitious root initiation, proliferation and incorporation of bio-reactor system in mountain ginseng (perennial plant found in the deep mountain) are achieved (Son *et al.*, 1999). Mountain ginseng adventitious roots could be used as healthy foods or tea. Large scale cultures of callus of yew (*Taxus* sp.) compound have been extensively investigated as an alternative source for taxol production (Furmanowa *et al.,* 1997; Yukimune *et al.,* 1996; Wickremesinhe and Arteca, 1994).

Siberian ginseng is found to effect invigoration, life extension and homeostasis in addition to showing therapeutic activity against hypertension, diabetes, cancer, inflammation, fever and pain, neuralgia, etc, as do mountain ginsengs. Siberian ginseng cultures produce some beneficial analogs which are interesting compounds that have not been previously reported from intact plants (data not shown). Moreover, plantlets can be used as healthy foods and medicinal materials.

REFERENCES

Choi YE, Soh WY. (1993) Structural aspects of somatic embryos derived from cultured zygotic embryos in *Acanthopanax senticosus* L. Korean J Plant Tissue Cult **20**, 261-266.

Choi YE, Kim JW, Soh WY. (1997) Somatic embryogenesis and plant regeneration from suspension cultures of *Acanthopanax koreanum* Nakai. Plant Cell Reports **17**, 84-88.

Furmanowa M, Glowniak K, Baranek KS, Zgorka G, Jozefczyk A. (1997) Effect of picloram and methyl jasmonate on growth and taxane accumulation in callus culture of *Taxus x media* var. Hatfieldii. Plant Cell Tissue Organ Cult **49**, 75-79.

Furuya T, Yoshikawa T, Orihara Y, Oda H. (1983) Saponin production in cell suspension cultures of *Panax ginseng*. Planta Med **48**, 83-87.

Green CE, Phillips RL. (1975) Plant regeneration from tissue cultures of Maize. Crop Sci **15**, 417-421.

Gui Y, Guo Z, Ke S, Skirvin RH. (1991) Somatic embryogenesis and plant regeneration in *Acanthopanax senticosus*. Plant Cell Reports **9**, 514-516.

Hibino K, Ushiyama K. (1996) Industrial production of ginseng by plant tissue culture technology. 5th Pacific Rim Biotechnol **6**, 136.

Kim JW, Soh WY. (1996) Plant regeneration through somatic embryogenesis from suspension cultures of *Allium fistulosum* L. Plant Sci **114**, 215-220.

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

Park HK. (1997) Morphology, germination and growth characteristics of kasiogalpi (*Eleutherococcus senticosus* Max.). a thesis for a doctorate, Chonbuk University, Korea.

Pence VC, Hasegawa PM, Janick J. (1980) Initiation and development of asexual embryos of *Theobroma cacao* L. in vitro. Zeitschrift fur Pflanzenphysiol **98**,

1-14.

Son SH, Choi SM, Hyung SJ, Yun SR, Choi MS, Shin EM, Hong YP. (1999) Induction and cultures of mountain ginseng adventitious roots and AFLP analysis for identifying mountain ginseng. Biotech Biopro Eng **4**, 119-123.

Yukimune Y, Tabara H, Higashi Y, Hara Y. (1996) Methyl jasmonate-induced overproduction of paclitaxel and baccatin III in *Taxus* cell suspension cultures. Nature Biotechnol **14**, 1129-1132.

Wickremesinhe ERM, Arteca RN. (1994) *Taxus* callus cultures: initiation growth optimization characterization and taxol production. Plant Cell Tissue Organ Cult **35**, 181-193.