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Production of Adventitious Ginseng Roots Using Biorectors

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ABSTRACT *Panax ginseng* is an important medicinal plant that has been used worldwide for geriatric, tonic, stomachic, and aphrodisiac treatments. Ginsenosides contained in the ginseng root are the main substances having active functions for human body. The price of ginseng is very expensive due to a complex process of cultivation, and the yield of ginseng is limited, which cannot meet the demand of the increasing market. Researchers have applied plant biotechnology to solve the problems but there are still things to be determined towards ginsenoside production by large-scale adventitious root culture. In this experiment, 5 to 20 liter bioreactors were employed to determine optimal conditions for adventitious root culture and ginsenoside production of *Panax ginseng*. Callus was induced from the ginseng root on MS agar medium containing 1.0 mg · L^{-1} 2,4-D and 0.1 mg · L^{-1} kinetin. Then the callus was cultured on MS agar medium supplemented with 2.0 mg · L^{-1} IBA, 0.1 mg · L^{-1} kinetin, and 30 g · L^{-1} to induce adventitious roots. The maximum root growth and ginsenoside production were obtained in 1/2 MS medium. 2.0 mg · L^{-1} naphthalene acetic acid resulted in greater root growth than 2.0 mg · L^{-1} indole-3-butyric acid. Ginsenoside content increased with 2.0 mg · L^{-1} benzyl adenin or kinetin. High concentrations of benzyl adenin (above 3.0 mg · L^{-1}) decreased the adventitious root growth and ginsenoside productivity. NH_4^+ inhibited the ginsenoside accumulation, while high concentrations of K^+, Mg_2^+, and Ca_2^+ increased it. NH_4^+ at 0.5 and 1.0 times of the normal amount in 3/4 SH medium resulted in the greatest biomass increase, but the highest ginsenoside productivity was obtained when NO_3^- was used as the sole nitrogen source in the medium. Most microelements at high concentrations in the medium inhibited the root growth, but high concentrations of $MnSO_4$ enhanced the root growth. Root dry weight increased with increasing sucrose concentrations up to 50 g · L^{-1}, but decreased from 70 g · L^{-1}. Ginsenoside productivity was maximized at the range of 20 to 30 g · L^{-1} sucrose. In the experiment on bioreactor types, cone and balloon types were determined to be favorable for both adventitious root growth and ginsenoside production. Jasmonic acid was effective for increasing ginsenoside contents and Rb group ginsenosides mainly increased. These results could be employed in commercial scale bioreactor cultures of *Panax ginseng*.

Key words: Adventitious root, ginsenoside, jasmonic acid, *Panax ginseng* C.A. Meyer

Introduction

Panax ginseng C.A. Meyer belongs to the Araliaceae family and is traditionally considered one of the most potent medicinal plants in the Orient. Ginseng has been used for centuries as a health tonic. It is an antibiotic and has therapeutic properties against stress and cancer. It is also thought to promote longevity. The most important active component in ginseng roots is ginsenoside. Up to date, more than 20 different ginsenosides have been identified (Lee et al. 1995).

The demand for ginseng roots and its extracts has increased over the past years. Ginseng plants collected from their natural habitats have become so scarce that a wild specimen may be sold for millions of dollars. Ginseng plants are cultivated in local farms throughout Korea and China since the cultivation is a long and laborious process and the cultivated ginseng roots are

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expensive commodities.

Ginseng is cultivated under artificial shade or simulated forest conditions to meet its requirements of about 30% of full sunlight (Persons 1995). Seeding is still a principal propagation method of ginseng, which takes about 4 to 6 years to harvest. In addition, disease control remains the central problem in commercial cultivation of ginseng. Ohh (1986) has quantified losses from diseases in Korea as follows: anthracnose (20%-47%), damping off (55-50%), root rot (1%-60%), phytophthora (2%-30%), and alternaria blight (10%-20%). Consequently, the commercial cultivation of ginseng has required the application of pesticides, which resulted in the serious problem of pesticide residues. No efficient methods were found to control the pesticide residues even many approaches have been concerned (Yu and Ohh 1995; Joy and Parke 1995). Another major problem in ginseng cultivation is that a second planting made in the same ground will fail, a phenomenon known as a replant disease. Growers recognize the problem and do not replant at the same site. This poses a problem of limited land base for cultivation in Korea. Ginseng replant disease is very complex (Li 1995; Yu and Ohh 1995) even though Ohh et al. (1992) confirmed that *Cylindrocarpon destructants* was the causal organism leading to replant problems. Due to these problems, the traditional cultivation method cannot meet the increasing demand of ginseng market, especially the demand of ginsenosides.

Proctor and Bailey (1987) reviewed in vitro culture of various tissues of ginseng and the production of ginsenosides from the cell or the tissue has been successful (Furuya et al. 1983; Inomata et al. 1993). For Commercial scale production, a large amount of ginsenosides must be produced from ginseng cells at one time. Choi et al. (1995) selected two ginseng cell lines, KGC 13 and KGC 15, for ginsenoside production and of these KGC 13 produced the highest level in 34 days. Akalezi et al. (1997) (1999) investigated the effects of initial sucrose concentration and inoculum size on cell growth and saponin production in suspension cultures of *P. ginseng*. Gubar et al. (1997) investigated callus growth and glycoside accumulation in ginseng callus culture under a long-term action of exogenous phytohormones. Zhong (1998) found that a high concentration of kinetin (7 mg · L^{-1}) seriously inhibited cell growth. The highest saponin content of 13.9% was achieved under 2.0 mg · L^{-1} IAA and 0.07 mg · L^{-1} kinetin. Nitrogen source also affects the production of ginsenosides and polysaccharides in ginseng cell culture. The maximum production of saponin and polysaccharide was achieved when nitrate was used as the sole N source (Liu and Zhong 1997; Zhong and Wang 1998). Optimal concentrations of phosphate are dependent on the purposes: 1.04 mM for cell growth and 0.42 mM for saponin production.

Cell and tissue differentiation is necessary for the synthesis of secondary metabolites in plant cells (Kutney 1998) and considering the fact, adventitious root culture might be more suitable for ginsenoside production compared to callus culture. Recently, commercial scale cultures of ginseng adventitious roots are tried using large-scale bioreactors (Seon et al. 1999 and Son et al. 1999a). Yet, more works are needed toward the commercial use: It is important to determine the optimal culture conditions for adventitious root growth and ginsenoside production in small-scale bioreactors, by which large-scale bioreactor culture of ginseng adventitious roots could be established.

Callus induction and proliferation of ginseng adventitious roots

For callus induction, ginseng root pieces were inoculated on MS medium supplemented with 1.0 mg · L^{-1} 2,4-D, 0.1 mg · L^{-1} kinetin, and 3% sucrose. In callus proliferation, 2.0 mg · L^{-1} IAA, 5.0 mg · L^{-1} IBA, or 0.5 mg · L^{-1} 2,4-D combined with 0.1 mg · L^{-1} kinetin were suitable, among which 5.0 mg · L^{-1} IBA and 0.1 mg · L^{-1} kinetin resulted in the greatest biomass increase. Then adventitious roots were induced from the callus on MS agar medium supplemented with 2.0 mg · L^{-1} IBA, 0.1 mg · L^{-1} kinetin and 3% sucrose. The roots induced were subcultured in 400 mL conical flasks containing 100 mL of MS liquid medium supplemented with 2.0 mg · L^{-1} IBA, 0.1 mg · L^{-1} kinetin and 3% sucrose until they reached certain biomass. Finally the roots were transferred to 5 liter cone type bubble bioreactors and cultured for 6 weeks.

Adventitious roots of mountain ginseng could also be obtained through the same process as in the case of ginseng. 2,4-D (2.0 mg · L^{-1}) was used for callus induction and IBA for adventitious roots initiation and prolif-

eration (Son et al. 1999b). Furuya et al. (1983a) found that 2,4-D was essential for callus growth of *Panax ginseng*, but high concentrations of 2,4-D (0.5 mg · L^{-1} or more) remarkably inhibited the growth. It can be concluded that 2,4-D is suitable for induction and growth of the callus, while IBA is favorable for induction and proliferation of the adventitious root in ginseng culture.

There are distinct stages in adventitious root initiation: a) induction of a new meristematic locus, b) early cell divisions, c) later cell divisions to form an organized and determined root meristem, d) development of the root by cell growth from the meristem. In our experiments, adventitious roots in bioreactors became inflated and looked like callus; perhaps there is a dedifferentiation stage and a redifferentiation stage in the process of proliferation of the adventitious roots.

Effect of medium salt strength on adventitious root growth and ginsenoside production

Maximum root biomass and growth rate were obtained in 1.0 MS medium, and low salt strengths (1/2 and 2/3 MS medium) were suitable for both root growth and ginsenoside productivity. High salt strength (2.0 MS medium) inhibited the growth of adventitious roots, which leaded to the high ginsenoside content but low ginsenoside productivity.

There are reports of the effect of salt strength on root growth and secondary metabolite production in other medicinal plants. In *Bupleurum falcatum*, 1.0 MS medium was efficient for both the adventitious root growth and saikosaponin production (Yamamoto and Kamura, 1997). Of the twelve basal media tested in mountain ginseng adventitious root study, WPM (Lloyd and McCown 1981) allowed relatively high rate of root induction capacity as well as growth of the newly induced adventitious roots (Son et al. 1999c). Further experiments should be carried out with other kinds of media to determine optimal salt strength.

Effects of plant growth regulators on adventitious root growth and ginsenoside production

Root growth enhanced by increasing IBA concentration: The best results in fresh weight, dry weight, and growth rate were obtained at 4.0 mg · L^{-1}. However, increased IBA decreased the percentage of root dry weight. In the case of NAA treatments, 2.0 mg · L^{-1} induced greatest root growth. When compared NAA to IBA at the same concentration, NAA resulted in greater root growth than IBA. Considering these results, 2.0 mg · L^{-1} NAA was determined to be optimal for the adventitious root growth. BA and kinetin concentrations also affected the root growth and ginsenoside production. Yet, no significant difference in root growth was observed between BA and kinetin, resulting in the greatest root growth in 2.0 mg · L^{-1} BA and kinetin.

Plant growth regulator is one the key factors to influence the biomass increase and secondary metabolites production. In safflower cell cultures, high concentration of auxin was required for the cell growth, while high concentration of cytokinin was favorable for red and yellow pigment production (Hanagata et al. 1994). In *Panax notoginseng* cell culture, Zhong (1998) found that a high concentration of kinetin (7 mg · L^{-1}) seriously inhibited cell growth, while a high concentration of IAA (10 mg · L^{-1}) combined with a low concentration of BA (0.1 mg · L^{-1}) increased saponin production. It can be concluded that the kinds and concentrations of growth regulators that are optimal for root growth and secondary metabolite production depend on plant species.

Changes in root growth and medium components as affected by addition of culture medium

Ginseng adventitious roots were grown in 20-liter bioreactors containing 4 liters culture medium for 6 weeks. To increase the root growth, the same amount of the medium was added in 3 weeks after culture. Fresh weight, dry weight, and growth rate of the adventitious roots remarkably increased after 6 weeks of culture, while ginsenoside content decreased.

Sucrose content and electric conductivity decreased right after feeding the medium, while fructose and glucose contents increased. All anion contents (Cl^-, NO_3^-, $H_2PO_4^-$, SO_4^-) decreased after feeding the medium but changes in cation contents were different among cations. In the case of cations, NH_4^+ was almost depleted in 3 weeks of culture before feeding the medium and

was consumed all after feeding the medium. These results indicated that a large amount of NH_4^+ is required for the root growth. There were little changes in Na^+ and K^+ during the whole culture period. Mg_2^+ and Ca_2^+ were absorbed gradually as in the case of anions. From the results, it could be concluded that sucrose and NH_4^+ were the key elements for ginseng adventitious root growth.

Effects of macro and microelements on adventitious root growth and ginsenoside Production

Normal MS medium was suitable for the adventitious root growth, but 1/2 or 3/4 MS medium was more suitable for ginsenodie production. NH_4^+ content in 3/4 SH medium resulted in high biomass growth, but it was harmful for ginsenoside synthesis. Highest ginsenoside content was obtained from SH medium without containing NH_4^+, indicating that NH_4^+ inhibited ginsenoside accumulation. Likewise, low concentrations of K^+, Mg_2^+, and Ca_2^+ were effective in biomass increase, while high concentrations of those elements were suitable for ginsenoside production.

Five or ten times the concentrations of $CoCl_2$, $CuSO_4$, KI, and $ZnSO_4$ in 3/4 SH medium inhibited the adventitious root growth. In the case of $MnSO_4$, 5 times concentration in the same medium increased the adventitious root growth, but 10 times decreased the root growth. Ginsensoide production was enhanced by 10 times concentrations of $CuSO_4$ and $ZnSO_4$, and 5 times concentration of $MnSO_4$ in the culture medium. However, KI and $CoCl_2$ inhibited both the adventitious root growth and ginsenoside production at 5 to 10 times high concentrations of the culture medium. Considering this, KI and $CoCl_2$ concentrations should be controlled at low levels in ginseng adventitious root culture.

The initial phosphate level in medium was claimed to be one of the most important factors influencing plant cell cultures. In suspension cultures of *Panax notoginseng*, both cell growth and saponin accumulation were greatly improved by increasing the initial phosphate concentration in the culture medium by 1.25 mM. However, phosphate concentrations more than 1.5 mM, gave a negative effect on saponin production.

The effect of ammonium/nitrate (NH_4^+/NO_3^-) ratios in MS medium on adventitious root growth and ginsenoside production was investigated. The result suggested that nitrate played an important role in biomass increase and ginsenoside production rather than ammonium. High NH_4^+ concentrations inhibited both root growth and ginsenoside production. Maximum ginsenoside productivity was obtained when nitrate was used as the sole N source and the greatest adventitious root biomass was obtained at a 1: 2 ratio of NH_4^+ to NO_3^-. 1: 2 NH_4^+/NO_3^- ratio also resulted in the second highest ginsenoside productivity. Similar results were obtained in *Panax quinquefolium* cell culture (Zhong and Wang 1998). They reported that a high growth rate and a great final cell biomass were obtained at low ratios of ammonium to nitrate, and the maximum production of saponin and polysaccharide was archived when nitrate was used as the sole nitrogen source. The maximum production of ginseng saponin and polysaccharide was obtained at 0:1 and 1:2 ammonium/nitrate ratios, respectively. In *Pinus strobus* cell culture, it was also claimed that high concentrations of ammonium inhibited callus and cell growth (Kaul and Hoffman 1993). In anthocyanin production by cell cultures of *Aralia cordata*, it was necessary to optimize nitrate to ammonium ratio for improving metabolite accumulation (Sakamoto et al. 1994). In cell culture of *Lithospermum erythrorhizon*, shikonin synthesis was inhibited by ammonium ions in the culture medium and the addition of nitrate was required at the end of the growth phase (Tabata and Fujita 1985).

As described above, nitrate and ammonium ions have different effects on cell cultures, as well as in tissue and organ cultures. Nitrate is known to promote secondary metabolite synthesis, while ammonium inhibits it. Therefore, it is very important to select optimal nitrogen sources and ammonium/ nitrate ratios according to plant species in the secondary metabolite production.

Effects of sucrose concentration and osmotic agent on the adventitious root growth and ginsenoside production

Sucrose is an important carbon source for plant cell and tissue cultures. It has been demonstrated that initial sucrose concentration can affect a number of cul-

ture parameters such as growth rate and yield of secondary metabolite in plant cell cultures. For example, in the cell suspension culture of *Perilla frutescens*, a relatively higher sucrose concentration (45 $g \cdot L^{-1}$) was favorable to anthocyanin production (Zhong et al. 1994; Zhong and Yoshida 1995). Zhang et al. (1996) reported that the initial sucrose concentration affected cell growth and saponin production in *Panax ginseng*. They obtained the maximum cell growth at an initial sucrose concentration of 30 $g \cdot L^{-1}$ and the maximum saponin production at initial sucrose concentrations of 6 to 8 $g \cdot L^{-1}$. The results of our experiment also confirmed the influence of initial sucrose concentrations on the adventitious root growth of *Panax ginseng*: Sucrose concentrations were varied at 10, 20, 30, 50, 70, and 90 $g \cdot L^{-1}$ to observe difference in the adventitious root growth. Root dry weight increased with increasing sucrose concentration up to 50 $g \cdot L^{-1}$, then decreased from a sucrose concentration of 70 $g \cdot L^{-1}$. Maximum fresh weight, dry weight, and growth rate of the roots were obtained at a 50 $g \cdot L^{-1}$ sucrose. Sucrose concentrations over 70 $g \cdot L^{-1}$ inhibited fresh and dry weight of the roots. The effect of sucrose concentrations on ginsenoside production is under investigation.

Osmotic agents (sorbitol and mannitol) also affected the root dry weight and the ginsenoside productivity. Treatments of osmotic agents increased the root growth but decreased the ginsenoside productivity. Root dry weight increased at 0.1 M mannitol or sorbitol but decreased with increasing those concentrations, resulting in half the dry weight at 0.5 M. Ginsenoside productivity was lowered by the addition of the osmotic agents. Particularly, a high concentration (0.5 M) of mannitol strongly inhibited ginsenoside production. Further experiments are required to select more suitable osmotic agents for both root growth and ginsenoside production.

The Effect of bioreactor types on the adventitious root growth and ginsenoside production

Roots were grown in 4 different types of bioreactor for 5 weeks: Cone type, balloon type, Bulb type, and Drum type. Cone and balloon type bioreactors were far more effective in the root growth and ginsenoside production compared to the other two types. Root biomass and ginsenoside contents were correlated with the amounts of salt elements and sucrose that were consumed during the culture: The higher uptake in salt elements and sucrose, the more increase in root biomass. There were differences in root biomass increase and ginsenoside accumulation during the culture. Root growth was slow for the first two weeks, and accelerated during the 3rd and the 4th week. After that, the root growth got into a stationary stage. On the other hand, ginsenoside content began to increase slowly after three weeks of culture and peaked at the fifth week. It was considered that the reason for differences in root growth and ginsenoside production according to bioreactor types was different type of air-flow imported into the bioreactors. The cells and organs cultured in bioreactors were strongly subjected by three forces: agitation-based laminar force, turbulent force, and bubble-based force (Namdev and Dunlop 1995). Likewise, ginseng adventitious roots cultured in different types of bioreactor were influenced by different air agitating environments. It has been confirmed that agitation in bioreactors affected cell aggregation and secondary metabolites production (Su 1995).

The effect of elicitors on ginsenoside production

Elicitor induced accumulation of secondary metabolites has received attention (Eilert 1987). An activation of monoterpenic indole alkaloid biosynthesis by jasmonates was reported in *Catharanthus roseus* and *Cinchona ledgeriana* seedlings (Aerts et al. 1994) and in cell suspensions of *Rauvolfia canescens* (Gundlach et al. 1992). In plant-pathogen interaction, jasmonates are powerful inducers of genes encoding pathogen protectants such as proteinase inhibitors (Herde et al. 1996), stress protectants such as osmotin (lehmann et al. 1995), or enzymes such as phenylalanine ammonialyase and chalcone synthase or 3-hydroxy-3-methylglutaryl Coenzyme A reductase (HMGR) (Maldonado-Mendoza et al. 1994). In C. roseus suspension cells cultured in a 2,4-D-starved medium, exogenous jasmonic acid greatly increased alkaloid production (Gantet et al. 1998).

In our experiment, jasmonic acid was used as an elicitor to increase ginsenoside content. Total ginseno-

side content lineally increased with increasing concentrations of jasmonic acid up to 10 mg · L^{-1}. Total ginsenoside content increased about 6 times by 10 mg · L^{-1} jasmonic acid compared to control. The effect of jasmonic acid appeared in an early stage of culture. For example, 2.0 mg · L^{-1} jasmonic acid resulted in 4.3 times increase in total ginsenoside content 7 days after treatment. Ginsenosides mainly increased were Rb group, while the contents of Rg group ginsenosides were kept stable. Among the ginsenosides, the contents of Rg b1, Rc, Rb2, and Rd increased more than those of others. It is required to experiment with other elicitors to select better elicitors for ginsenoside production.

Conclusion

Ginseng adventitious root culture has advantages in ginsenoside production over cell or callus cultures. Especially, application of bioreactor systems in the adventitious root culture could make it possible to produce ginsenoside in commercial scale. Bioreactor culture system is more advanced than the traditional tissue culture system as the culture conditions in bioreactors can be optimized by on-line manipulation of the culture conditions, e.g., temperature, pH and the concentrations of dissolved oxygen, carbon dioxide, nutrients and so forth. Nutrient uptake can also be enhanced by continuous medium circulation, which ultimately increased cell proliferation rate. In this paper, we introduced effects of culture conditions on ginseng adventitious root growth and ginsenoside production in bioeactors. It could be possible to establish commercial scale bioreactor systems in ginsenoside production if further experiments are carried out with detailed factors.

Refererences

Aerts RJ, Gisi D, De Carolis E, De Luca V, Baumann TW (1994) Methyl jasmonate vapor increase the developmentally controlled synthesis of alkaloids in *Cartharanthus* and *Cinchona* seedlings. Plant J **5**: 635-643

Akalezi CO, Liu S, Li QS, Yu JT, Zhong JJ (1999) Combined effects of initial sucrose concentration and inoculum size on cell growth and ginseng saponin production by suspension cultures of *Panax ginseng*. Process Bioche **34**: 639-642

Choi KT, Lee CH, Ahn IO, Lee JH, Park JC (1995) Characteristics of the growth and ginsenosides in the suspension-cultured cells of Korean ginseng (*Panax ginseng* C. A. Meyer). In: Bailey WG, Whitehead C, Proctor JTA and Kyle JT (eds), Proc Int Ginseng Conf Vancouver 1994, Canada pp: 259-269

Eilert U (1987) Elicitation: methodology and aspects of application. In: Constabel F, Vasil IK (eds), Cell Culture and Somatic Cell Genetics of Plants, Academic, San Diego Vol, **4**: 153-196

Furuya T, Yoshikawa T, Ishii T, Kajii K (1983a) Effects of auxins on growth and saponin production in callus cultures of *Panax ginseng*. Planta Med **47**: 183-187

Gantet P, Imbault N, Thiersault M, Doireau P (1998) Necessity of a functional octadecanoic pathway for indole alkaloid synthesis by *Catharanthus roseus* cell suspensions cultured in an auxin-starved medium. Plant Cell Physiol **39**: 220-225

Gubar SI, Gulko TP, Kunakh VA (1997) Growth and glycoside accumulation in ginseng callus tissue culture under a long-term phytohormones. Russian Journal of Plant Physiology **44**: 83-89

Gundlach H, Muller MJ, Kutchan TM, Zenk MH (1992) Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proc Natl Acad Sci USA 89: 2389-2393

Hanagata N, Ito A, Takeuchi T, Karube I (1994) Effect of medium uesd in first stage culturing on red pigment formation in suspension culture of Carthamus tinctorius. Journal of Biotechnology **34**: 213-216

Herde O, Atzorn R, Fisahn J, Wasternack C, Willmitzer L, Pena-Cortes H (1996) Localized wounding by heat initiates the accumulation of proteinase inhibitor II in abscissic acid deficient plants by triggering jasmonic acid biosynthesis. Plant Physiol **112**: 853-860

Inomata S, Yokoyama M, Gozu Y, Shimizu T, Yanagi M (1993) Growth pattern and ginsenoside production of *Agrobacterium*-transformed Panax ginseng roots. Plant Cell Rep **12**: 681-686

Joy AE and Parke JL (1995) Biocontrol of Alternaria leaf blight on American ginseng by bukholderia cepacia AMMD pp 93-100. IN: WG Bailey, C. Whitehead, JTA proctor, and JT kyle (eds.), Proc Int Ginseng Conf Vancouver 1994, Canada

Kaul K, Hoffman SA (1993) Ammonium ion inhibition of *Pinus strobus* L. callus growth. Plant Science 88: 169-173

Kutney JP (1998) Biotechnology and synthetic chemistry routes to clinically important compounds. Pure and Application Chemistry **70**: 2093-2100

Lee HS, Kim SW, Lee KW, Eriksson T, Liu JR (1995) *Agrobacterium*-mediated transformation of ginseng (*Panax ginseng*) and mitotic stability of the inserted beta-glucuronidase gene in regenerates from isolated protoplasts. Plant Cell Rep **14**: 545-549

Lehmann J, Atzorn R, Bruckner C, Reinbothe S, Leopold J,

Wasternack C, Parthier B (1995) Accumulation of jasmonates, abscissic acid, specific transcripts and proteins in osmotically stressed barely leaf segments. Planta **197**: 156-162

Li TSC (1995) The effects of chemical and organic treatment on ginseng seedlings planted in old ginseng soil pp 201-204. IN: WG Bailey, C. Whitehead, JTA Proctor, and JT Kyle (eds.), Proc Int ginseng Conf. Vancouver 1994, Canada

Liu S, Zhong JJ (1997) Simultaneous production of ginseng saponin and polysaccharide by suspension cultures of *Panax ginseng* - nitrogen effects. Enzyme and Microbial Technology **21**: 518-524

Lloyd GB, McCown BH (1981) Commercially feasible micropropagation of mountain laurel (*Kamlmia latifolia*) by use of shoot tip culture. Prop Int Plant Proc Soc **30**: 421-427

Maldonado-mendoza I, Burnett RJ, Lopez-meyer M, Nessier CL (1994) Regulation of 3-hydroxy-3-methylglutaryl-coenzyme A reductase by wounding and methyl jasmonate. Plant Cell Tissue Organ Cult **38**: 351-356

Namdev PK and Dunlop AE (1995) Shear sensitivity of plant cells in suspensions, present and future. Applied Biochemistry and Biotechnology **54**: 109-131

Ohh SH (1986) Diseases of ginseng: environmental and host effect on disease outbreak and growth of pathogens. Kor J Ginseng Sci **5**: 73-84

Ohh SH, Yu YH, Kim KH, and Cho DH (1992) Studies on control of soil-brone diseases and insects of ginseng and development of antifungal compound. pp 121-184. In: Koera Ginseng and Tobacco Research Inst., Ginseng Cultivation Bul

Persons, WS (1995) American ginseng farming in its woodland habitat. pp 78-83. In: WG Bailey, C. Whitehead, JTA Proctor, and JT Kyle (eds.), Proc Int Ginseng Conf Vancouver 1994, Canada

Preil W (1991) Application of bioreactors in plant propagation. In: Debergh PC and Zimmerman RH (eds), Micropropagation, Kluwer Academic Publisher, Netherlands pp 425-445

Proctor JTA, Bailey WG (1987) Ginseng: industry, botany, and culture. Hort Rev **9**: 188-236

Sakamoto K, Iida K, Sawamura K, Hajiro K, Asada Y, Yoshikawa T, Furuya T (1994) Anthocyanin production in cultured cells of *Aralia cordata* thunb. Plant Cell, Tissue and Organ Culture **36**, 21-26

Seon JH, Yu KW, Cui YY, Kim MH, Lee SJ, Son SH, Paek KY (1999) Application of bioreactor for the production of saponin by adventitious root cultures in *Panax ginseng*. In: Altman A (ed), Plant Biotechnology and *In Vitro* Biology in the 21st Century, Kluwer Academic Publishers, Netherlands pp 329-332

Son SH, Choi SM, Yun SR, Kwon OW, Lee OW, Paek KY (1999a) Large-scale culture of plant cell and tissue by bioreactor system. J Plant Biotechnology **1**: 1-7

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

Son SH, Choi SM, Lee DS, Yun SR, Paek KY (1999c) Commercial application of mountain ginseng through bioreactor culture system. In: Paek KY (ed), Proceedings of the Korea-Japan joint symposium on transplant production in horticultural plants, Chungbuk Natonal University, Cheongju, Korea pp 9-14

Su WW (1995) Bioprocessing technology for plant cell suspension cultures. *Applied Biochemistry and Biotechnology* **50**: 189-230

Tabata M, Fujita T (1985) Producing of shikonin by plant cell cultures. In: Zaitlin M, Day P, Hollaender A (eds), Plant Science: Relevance to Agriculture in the Eighties, Academic Press, New York pp 207-218

Yamamoto O, Kamura K (1997) Production of saikosaponin in cultured roots of *Bupleurum falcatum* L. Plant Tissue Culture and Biotechnology **3**: 138-147

Yu YH, Ohh SH (1995) Problems and present status of research on ginseng diseases in Korea. pp 120-130. In: WG Bailey, C. Whitehead, JTA Proctor, and JT Kyle (eds.), Proc Int Ginseng Conf Vancouver 1994, Canada

Zhang YH, Zhong JJ, Yu JT (1996) Enhancement of ginseng saponin production in suspension cultures of *Panax notoginseng*: manipulation of medium sucrose. J Biotechnol **51**: 49-56

Zhong JJ (1998) Production of ginseng saponin and polysaccharide by cell cultures of *Panax notoginseng* and *Panax ginseng*: Effects of plant growth regulators. Applied Biochem and Biotech **75**: 261-268

Zhong JJ, Wang SJ (1998) Effects of nitrogen source on the production of ginseng saponin and polysaccharide by cell cultures of *Panax quinquefolium*. Process Biochemistry **33**: 671-675

Zhong JJ, Yoshida (1995) High-density cultivation of *Perilla frutescens* cell suspensions for anthocyanin production effects of sucrose concentration and inoculum size. Enzyme Microb Technol **17**: 1073-1079

Zhong JJ, Xu GR, Yoshida T (1994) Effect of initial sucrose concentration on excretion of anthocyanin pigments in suspended cultures of *Perilla frutescens* cells. World J Microbiol Biotechnol **10**: 590-592