A THESIS

FOR THE DEGREE OF MASTER OF SCIENCE

Improvement of ginsenoside content in Korean Wild Ginseng (*Panax schinseng* Nees) root cells induced by γ-irradiation

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A thesis submitted in partial fulfillment of the requirement for the degree of master of science.

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This thesis has been examined and approved.

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요약문

산삼(*Panax schinseng* Nees)은 아시아에서 의약품과 건강보조제 등으로 가장 널리 사용되는 것 중 하나이다. 산삼의 성분 중에서 사포닌은 30 종이상 함유되어 있고, 사포닌의 효능은 체력강화와 면역력증가 등 여러가지 효과가 있다. 본 연구는 산삼의 부정근에 사포닌 함량을 증가 시키기 위해서 감마선 조사를 수행하였다. 돌연변이 유도를 위한 ^{60}CO 감마선 조사 선량은 5, 10, 25, 50, 75, 100, 200Gy 로 산삼의 부정근에 각각 처리하였고, 각각의 조사선량에서 생존한 개체로부터 돌연변이체를 선발하였다. 산삼 세포주는 삼각플라스크와 바이오리액터에서 30 일간 배양하여 선발하였다. 돌연변이체 선발기준은 산삼세포주 뿌리의 2 차근수, 길이, 직경, 생장율 및 조사포닌 함량의 5 가지 요인으로 대조구와 비교하여 선발되었다. 진세노사이드 Rg1, Re, Rb1, Rb2 함량은 HPLC-PDA 검출기로 측정하였고, 검량선법으로 정량하여 대조구와 비교하였다. 산삼의 부정근에 방사선을 조사한결과 50Gy 이하에서는 형태적 특성이 대조구와 유사하였다. 50Gy 이상의 선량을 처리한 부정근에서는 생리적 장애로 인한 성장억제 및 갈변현상이 발생하였고, 형태적 특성이 대조구와 차이가 있는 4 개의 세포주를 선발하였다. 삼각플라스크에서 배양한 세포주의 형태적 특성과 조사포닌 함량을 비교한 결과 세포주 1은 대조구보다 2차근 수, 길이와 생장율은 낮았지만 대조구에 비해서 직경이 31.3%, 조사포닌이 40.6%증가하여 다른 세포주에 비해서 가장 높게 나타났다. 4 개의 진세노사이드 (Rg1, Re, Rb1, Rb2) 총함량은 세포주1이 대조구의 진세노사이드 함량보다 60% 높게 나타났다. 바이오리액터에서 배양한 세포주들의 형태적 특성과 조사포닌 함량을 비교한 결과 세포주 1 의 2 차근 수와 길이는 삼각플라스크 배양에서와 유사한 형태적 특성이 관찰되었다. 반면에 생장율은 대조구와 유사하였고, 조사포닌 함량은 87.3%로 삼각플라스크에서 보다 2.2 배이상 증가했다. 4 개의 진세노사이드 총함량은 세포주 1이 대조구의 진세노사이드 함량보다 126%로 삼각플라스크에서 보다 2.1 배 증가하였다.

결과적으로 삼각플라스크와 바이오리액터에서 배양한 4 개의 세포주 중에서 조사포닌 함량이 가장많은 돌연변이세포주 1 은 2 차근 수와 길이가 대조구 보다 낮았으나, 뿌리의 직경과 사포닌함량이 높게 나타났다. 또한 바이오리액터 배양은 삼각플라스크에 비해서 생장율과 진세노사이드 함량이 높게 나타났다. 바이오리액터는 공기의 순화 및 영양배지의 공급이 일정하기때문에 삼각플라스크에서 보다

생육환경이 적합하여 산삼의 부정근 배양에 유용한 배양시스템으로 사료된다. 본 연구에서 수행한 산삼의 부정근에 방사선 조사방법은 사포닌 함량이 증가된 돌연변이 산삼 세포주을 유도하는데 효과적이었다. 방사선 처리에 의해서 유도된 돌연변이 세포주는 2 차 대사산물의 함량변화와 관련된 유전자 규명에 도움이 될 수 있을 것으로 기대된다.

ABBREVIATIONS

NAA	Naphthalene acetic acid
IAA	Indole-3-acetic acid
HPLC-PDA	High performance liquid chromatograph-photodiode array detector
ACN	Acetonitrile
MS	Murashige & Skoog
FW	Fresh weight
DW	Dry weight
PD	Panaxadiol
PT	Panaxatriol
MJ	Methyl jasmonate
UV	Ultraviolet
Gy	Gray

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SUMMARY

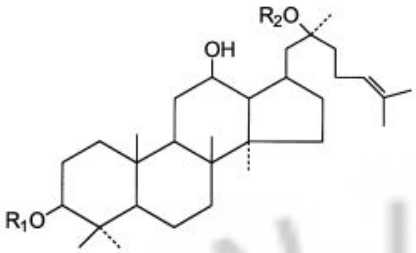
Panax schinseng Nees (Korean wild ginseng) is one of the oldest and the most widely used herbal medicines for the oriental people, more than 30 ginsenosides were identified. In order to evaluate effects of γ-irradiation on adventitious root growth and ginsenoside content, adventitious roots of *Panax schinseng* were treated with γ-ray of 5, 10, 25, 50, 75, 100 and 200Gy to induce mutation. Cell lines were cultured in flask and bioreactor for 30 days and selected based on the length, number, growth ratio, diameter and crude saponin content of the irradiated adventitious roots. The contents of ginsenosides Rb1, Rb2, Rg1, and Re in wide type and selected cell lines were evaluated by HPLC-PDA detector with digoxin as the internal standard. After irradiation, below 50Gy, the morphology and crude saponin content of irradiated adventitious root were similar with that of non-irradiated adventitious root. Otherwise, above 50Gy, the morphology and crude saponin content were different. Therefore, the dosage over 50Gy was considered as adequate for the selection of mutant cell lines of schinseng. After growth 30 days in flask, 4 cell lines were selected according to the the morphology and crude saponin content. In cell line 1, the values of secondary root number, length and growth ratio were decreased, but the values of secondary root diameter and crude saponin were increased 31.3%, 40.6% respectively compared to control. Total content of 4 ginsenosides was increased 60% compared to control. In bioreactor, the values of secondary root number and length were decreased, growth ratio was similar with that of control. The value of secondary root diameter and crude saponin in bioreactor were increased 22.7%, 87.3% respectively compared to control. Total content of 4 ginsenosides were increased 126% repectively compared to control. Although the values of secondary root number and length were lower than control in flask and bioreactor, interestingly, the diameter of secondary root increased compared with that of control. The value of growth ratio was similar with that of control in bioreactor, increased compared with that of flask. Crude saponin content in bioreactor was increased by 2.2 times that of the flask, ginsenoside content was increased 2.1 times that of the flask. In this study, we selected cell lines with a

significantly increased biomass productivity and ginsenoside content in bioreactor culture compared with that of flask culture, probably due to their better nutrient status and low-stress environments.

Based on the results obtained in this report, gamma irradiation is powerful and useful tool for the enhancement of production of secondary metabolites and for the bioreactor culture system, especially for ginsenoside. This result suggests that gamma irradiation might have triggered the enzyme activities for the synthesis of ginsenosides. Further work will be required to investigate the related enzymes involved in the enzymatic biosynthesis of ginsenosides.

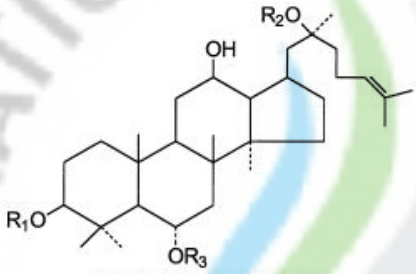
INTRODUCTION

There are several medicinal *Panax* species identified from all over the world. From a number of speices, *Panax schinseng* (Korean wild ginseng) is one of the oldest and the most widely used herbal medicines and this species is almost extinct in wild habitats. Fortunately, *Panax schinseng* is widely cultivated in Korea, China, Japan, and several countries in North America and Europe under special shade conditions. (Hobbs 1996, Mabberley 1987). Depending on the species, growth condition and location etc, the ginsenoside contents vary widely which act on the central nervous system, cardiovascular system, endocrine system, and immune system. These active ingredients also increase endocrine secretion, promote immune function, and have anti-aging and stress relieving effects. *Panax ginseng* (Oriental ginseng) and *Panax quinquefolius* (American ginseng) are characterized well by phytochemistry (Mallol *et al*. 2001, Ngan *et al*. 1999) and more than 30 ginsenosides were identified from this genus. They can be classified into three groups based on their aglycones (Figure 1): the protopanaxadiol-type, protopanaxatriol-type, and oleanane type saponins (Jung *et al*. 2003, Nah 1997).



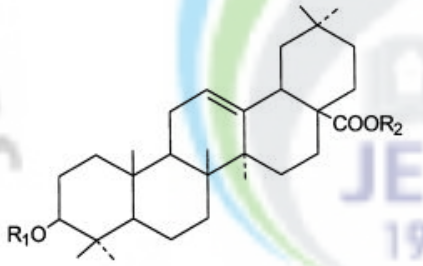
(20S)-Protopanaxadiol
(R_1=R_2=H)

Ginsenoside	R_1	R_2
Rh_2	Glc	H
Rg_3	Glc^2-Glc	H
Rd	Glc^2-Glc	Glc
Malonyl-Rd	Glc^2-Glc^6-Ma	Glc
Rb_2	Glc^2-Glc	Glc^6-Arap
Malonyl-Rb_2	Glc^2-Glc^6-Ma	Glc^6-Arap
Rc	Glc^2-Glc	Glc^6-Araf
Malonyl-Rc	Glc^2-Glc^6-Ma	Glc^6-Araf
Rb_1	Glc^2-Glc	Glc^6-Glc
Malonyl-Rb_1	Glc^2-Glc^6-Ma	Glc^6-Glc
Rb_3	Glc^2-Glc	Glc^6-Xyl
Ra_1	Glc^2-Glc	Glc^6-$Arap^4$-Xyl
Ra_2	Glc^2-Glc	Glc^6-$Araf^6$-Xyl
Ra_3	Glc^2-Glc	Glc^6-Glc^3-Xyl



(20S)-Protopanaxatriol
(R_1=R_2=R_3=H)

Ginsenoside	R_1	R_2	R_3
Rh_1	H	H	Glc
Rg_1	H	Glc	Glc
Rf	H	H	Glc^2-Glc
20-Glc-Rf	H	Glc	Glc^2-Glc
Rg_2	H	H	Glc^2-Rha
Re	H	Glc	Glc^2-Rha



Ginsenoside Ro

R_1	R_2
Glc^2-Glc	Glc

Glc= β-D-glucose Rha= α-L-rhamnose
Arap= α-L-arabinose (pyranose)
Araf= α-L-arabinose (furanose)
Xyl= β-D-xylose Ma= malonyl

Figure 1. Structures of ginsenosides isolated from *Panax ginseng*.

The both species are used for preparation of tonic, prophylactic and anti-ageing agents (Chang & But 1986). Some ginsenosides are used widely, such as Rg1, Re, Rb1 are used to determinate ginseng quality (Wu *et al*. 2007). Some ginsenosides, like Rg3, Rh2 and Rh1 are difficult detected in cultured ginseng. Ginsenoside Rb1, Re, Rg1 and Rb2 were the precursors to form other ginsenosides by Human Intestinal Microflora and steaming. Different forms of ginsenosides were showed several uses.

Ginsenoside Rg1 Ginsenoside Rg1 inhibits the rat brain cAMP phosphodiesterase activity, excitatory amino acids are involved in neuronal survival, synaptogenesis, neuronal plasticity, learning and memory processes, estrogen-like activity, and promoted functional neovascularization into a polymer scaffold in vivo (Stancheva *et al*. 1993, Balazs *et al*. 1998, Muller *et al*. 1998, Chan *et al*. 2002, Sengupta *et al*. 2004).

Ginsenoside Rb1 Ginsenoside Rb1 successfully improved homocysteine-induced reduction of endothelial nitric oxide synthase expression and reduced homocysteine-induced oxidative stress (Zou *et al*. 2005). Preconditioning of Rb1 showed protective effects on myocardial ischemia and reperfusion injury (Wang *et al*. 2008). Rb1 promoted neurotransmitter release (Xue *et al*. 2006) and used to prevent or treat gastritis and gastric ulcers (Jeong *et al*. 2003).

Ginsenoside Re Some reports revealed that both Rb1 and Re decreased cardiac contraction in adult rat ventricular myocytes (Scott *et al*. 2001). Some reports says that treatment of both Rg1 and Re could be a novel group of nonpeptide angiogenic agents and may be useful for tissue regeneration (Yu *et al*. 2006). Compared with other gingenosides Re showed anti-diabetic activities (Xie *et al*. 2005) and free radical scavenging properties (Xie *et al*. 2006).

Ginsenoside Rb2 The another form of gingenoside Rb2 is reported to stimulate protein and RNA synthesis and increases RNA polymerase activity (Yokozawa *et al*. 1993a, 1993b; Yokozawa *et al*. 1990). Rb2 accelerates would healing effects like cell proliferation, expression of proliferation related factors, and epidermis formation (Choi 2002). Rb2 can be used as anti-tumor or chemopreventive agents, especially acting on cancer promotional stage

(Kang *et al*. 2000).

Ginsenoside Rc Ginsenoside Rb2 and Rc may have effects that prevent or limit the development of breast cancer, affects the motility of sperm (Chen *et al*. 2001, Lee *et al*. 2003).

Ginsenoside Rd Ginsenoside Rd inhibiting 26S proteasome activity. Ginsenoside-Rd has been proved to decrease the severity of renal injury induced by cisplatin. Rd is Cytotoxic towards HeLa Cancer Cells and Induces Apoptosis (Chang *et al*. 2008, Yokozawa *et al*. 2000, Yang *et al*. 2006).

Ginsenoside Rh2 Ginsenoside Rh2 has the anti-fatigue, anti-cancer effect, antiallergic and reduces ischemic brain injury in rats (Oh *et al*.1999, Nakata *et al*. 1998, Park *et al*. 2003, Park *et al*. 2004).

Ginsenoside Rh1 Ginsenoside Rh1 and Rh2 inhibit the induction of nitric oxide synthesis in murine peritoneal macrophages. G-Rh1 acts as a functional estrogenic ligand In MCF-7 cells. Ginsenoside Rh1 possesses antiallergic and anti-inflammatory activities (Park *et al*. 1996, Lee *et al*. 2003, Park *et al*. 2004).

Ginsenoside Rg3 Ginsenoside Rg3 significantly inhibited growth and angiogenesis of ovarian cancer and inhibition of *in vitro* Tumor Cell Invasion (Xu *et al*. 2008, Shinkai *et al*. 1996). Ginsenoside Rg3 is a body function balancer, long term taking Rg3 can get anti senility effects. Because ginsenoside Rg3 can invigorate blood circulation and improve brain and body activity. It has obvious effects for anti wrinkle, would make skin fresh, bright and glossy. Ginsenoside Rg3 has obvious function for body tonic, improve muscle tone and prevent fatigue.

Due to unavoidable limitations in *Panax* breeding, it is difficult to produce large amounts of roots under field conditions (Han *et al.* 2009). Hence, several scientists followed different biotechnological methods such as root culture, *agrobacterium*-mediated hairy root production, bioreactor mediated large scale production. Jasmonic acid has been shown to be an effective elicitor for secondary metabolite induction in plant cell cultures (Ketchum *et al.* 1999). Recently, MJ (Methyl jasmonate) mediates the reprogramming of cellular metabolism

and cell cycle progression via the regulation of jasmonic acid biosynthesis (Pauwels *et al.* 2008). The positive effect of MJ on ginsenoside production from ginseng cell suspension, hairy root and adventitious root cultures has been previously documented (Lu *et al.* 2001, Palazón *et al.* 2003, Choi *et al.* 2005, Bae *et al.* 2006). Mutation breeding is considered as one of the effective plant breeding methods for improved variety of crop production. Among several methods, γ-irradiation was used in several species for crop improvement program. Recent report showed that mutagenesis by γ-irradiation gained rapid development in enhanced ginsenoside production of *Panax ginseng*. Callus were used for gamma irradiation and the cell lines selected from dosage 30Gy-treatment and ginsenoside content was significantly increased (Kim *et al*. 2009). Suspension culture of *Lithospermum erythrorhizon* cells was irradiated by gamma irradiation and significantly stimulated the shikonin biosynthesis of the cells and increased the total shikonin yields by 400% at 16Gy (Chung *et al.* 2006). For maize, grain yield for irradiated samples is increased to levels above the unirradiated yield at doses up to about 250Gy with the optimum yield occurring at 150Gy. The corresponding increase for groundnut is observed at doses up to about 930Gy with optimum yield at a dose of 300Gy (Mokobia *et al*. 2006). The effect of gamma radiation on growth and yield of barley at different nitrogen levels, grain yield with maximum production at 100Gy (Subhan *et al.* 2004). γ-irradiation mediated mutagenesis of whole plants as well as roots yielded significant variations among the different mutated species, and 50Gy of γ-rays was determined to be the optimal dose for inducing mutations (Joseph *et al*. 2004).

Our present work is to investigate the effects of gamma irradiation on ginsenoside contents of schinseng adventitious root and to provide valuable information on the utilization of beneficial effects of gamma irradiation in root cell culture system of *Panax schinseng*.

MATERIALS AND METHODS

Plant material

Panax schinseng Nees is Korean wild ginseng. Wild ginseng grows naturally within its natural habitat conditions (Figure 2). Adventitious roots were induced and proliferated from wild *Panax schinseng* root and cultured in MS medium supplemented with NAA and IAA.

Figure 2. Korean wild ginseng (*Panax schinseng* Nees).

Establishment of adventitious root cultures

Panax schinseng adventitious roots were proliferated on MS (Murashige and Skoog 1962) medium as follows (mg/l): NH_4NO_3 (687.5), KNO_3 (1900), KH_2PO_4 (170), H_3BO_3 (6.2), $MnSO_4.4H_2O$ (23.3), $ZnSO_4.7H_2O$ (8.6), KI (0.83), $Na_2MoO_4.2H_2O$ (0.25), $CuSO_4.5H_2O$ (0.025), $CoCl_2.6H_2O$ (0.025), $CaCl_2.2H_2O$ (440), $MgSO_4.7H_2O$ (370), Na_2EDTA (37.3), $FeSO_4.7H_2O$ (27.8), Nicotinic acid (5), Pyridoxine Hydrochloride (1), Thiamine Hydrochloride (2.5), Glycine (2). Supplemented with 10.87 μmol Naphthalene acetic acid

(NAA), 1.43 μmol Indole-3-acetic acid (IAA) and 5% sucrose. The pH of the medium was adjusted to 6.0 before autoclaving at 121 ℃ and 1.2 Kgf/cm^2 pressure for 15 min.

Petridish culture condition

Adventitious roots were cut into 10 pieces, each 1-2 cm and cultured in petridishe(10 cm in diameter and 1.5 cm in height) with lid containing 50 ml MS solid medium was sealed with a wrap (Advantec, USA) and cultured at 23 ± 2 ℃ under dark condition. Adventitious roots were sub-cultured every 30 days.

Flask culture condition

Fresh roots (0.8 g) were inoculated into a 100 ml erlenmeyer flask containing 50 ml MS liquid medium with 10.87 μmol NAA, 1.43 μmol IAA and 5% sucrose. Cultures were shaken at 120 rpm in the light at 23 ± 2 ℃ for 30 days.

Bioreactor culture condition

The bioreactors were maintained at 23 ± 2 ℃ in a light condition until harvest. Adventitious roots were sub-cultured every 30 days in 15-L bioreactor (Biopia, Korea) containing 5-L MS medium. Fresh weight (15 g) of adventitious roots was inoculated into bioreactor for proliferation. After 30 days, the proliferated adventitious roots were used as explants for further experiments. The culture system showed in figure 3.

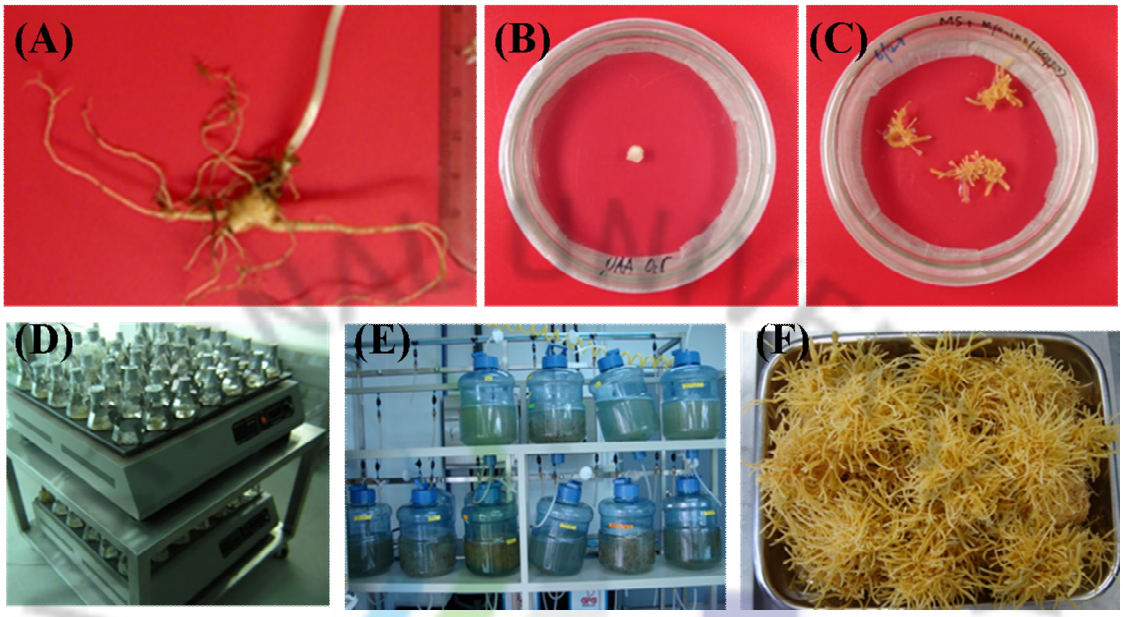


Figure 3. Culture system of *Panax schinseng* Nees. (A) Root of *Panax schinseng*; (B) Explant of schinseng root; (C) Adventitious root proliferation; (D) Adventitious root culture in flask; (E) Adventitious root culture in bioreactor; (F) Harvest.

Gamma irradiation

Adventitious roots (1-2 cm) were placed in plastic petridishes (10 pieces adventitious roots per pertridish), grown at 23 ℃ and cultured for 5 days in MS medium with 10.87 μmol NAA, 1.43 μmol IAA and 5% sucrose. They were exposed to gamma radiation from cobalt (^{60}Co) source using a γ-radiation apparatus at the Applied Radiological Science Research Institute, Jeju National University. Irradiation dosages were 0 (non-irradiated), 5, 10, 25, 50, 75, 100 and 200Gy. For each dosage, 2 petridishes of the samples were exposed in triplicate. Effects of gamma irradiation on adventitious root culture was evaluated by measuring the number of survival main roots after 5 weeks in the adventitious root solid culture medium.

Cell line selection in suspension culture

Secondary roots of survival main roots were transferred into 50 ml suspension culture MS medium with NAA and IAA in flask. According to the secondary roots number, length, diameter, growth ratio and crude saponin content, 4 cell lines were selected. The 4 cell lines were cultured in 15-L bioreactor with 5-L MS medium at last.

Determination of root weight, growth ratio, secondary root number, length and daimeter

Fresh weight (FW) and dry weight (DW) were measured after 10 days, 20 days and 30 days growth in flask and bioreactor. Root FW and DW were determined as follows. Roots were separated from the medium by passing through a 1 mm stainless steel sieve. Root FW was measured after rinsing once with tap water and blotting away surface water and root DW was recorded after roots were dried to a constant weight at 75 ℃ for several days (Kim *et al.* 2004). Root growth ratio was calculated by using the following formula (Yu *et al.* 2002):

$$\text{Growth ratio} = \frac{\text{Harvested DW (g)}}{\text{Inoculated DW (g)}}$$

After 10 days, 20days and 30 days growth, the length of secondary roots were measured by using the calipers. The number of secondary roots were counting according to the secondary roots of main adventitious roots. The diameter of secondary roots were measured with microscope and treatment by acetocarmine solution.

Extraction and determination of crude saponin

Extraction and determination of ginsenosides were carried out by modifying the method of Kwon *et al*. (2003). Ultrasound-assisted extraction was performed with a (Branson ultrasonics, USA). The output power is 117 volts and the frequency is 50/60 Hz. Extraction of crude saponin from adventitious roots of different cell lines was conducted as follows (Figure 4). Sample powder 1.00 g was placed into a 100 ml conical flask, into which 30 ml of 80% (v/v) methanol–water were added. Then the flask was sonicated for 1 h in an ultrasonic water bath. The extract obtained was evaporated using a rotary evaporator under vacuum at 55 ℃. The evaporated residue (total extract yield) was dissolved in 20 ml of distilled water and washed twice with 20 ml of diethyl ether to remove the fat contents using a separatory funnel. The aqueous layer was extracted four times with 20 ml of water-saturated n-butanol. The butanol solution was washed twice with 30 ml of distilled water to remove the impurities, thereby obtaining crude saponins. The remaining butanolic solution was transferred to the tarred round bottom flask for the evaporation using a rotary evaporator under vacuum at 55 ℃. All remaining steps and calculation of the percentage of crude saponins in the sample were done as per the determination of total extract yield.

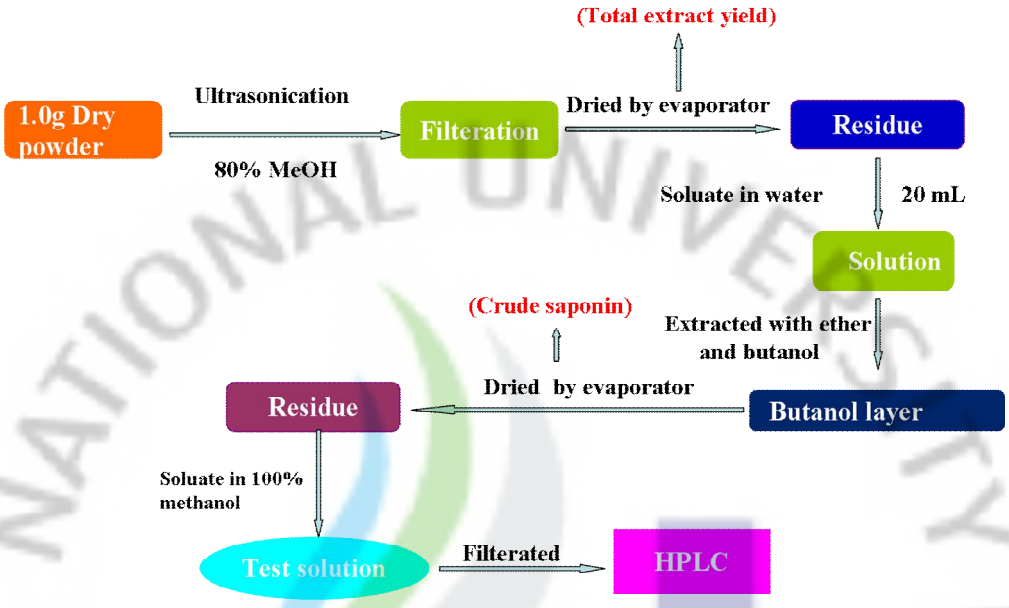


Figure 4. Extraction procedures of crude saponin in *Panax schinseng* Nees.

Determination of ginsenoside Rg1, Re, Rb1, Rb2 with HPLC

For ginsenoside assay, The HPLC conditions for ginsenoside isolation were modified of Park *et al*. (2007). Quantitative determinations were achieved by HPLC using a Capcell-pak C18 MG (4.6 × 250 mm) column (Shiseido, Japan), Waters 2998 Photodiode Array Detector, Waters 2690 Separations Module and Empower Program (Table 1).

Table 1. HPLC conditions for ginsenosides analysis.

Parameter	**Condition**
Instruments	Waters 2690 Separations Module Empower Program
Column	Capcell-pak C18 MG (4.6 × 250 mm) column, 5 ㎛ (Shiseido, Japan)
Mobile phase	Distilled water and Acetonitrile
Flow rate	1ml/min
Detector	Wavelength: 203 nm (PDA)
Scan wavelength	192 - 400 nm
Column temperature	35℃
Sample injection	10 µl
Run time	60 min

The solvents used were of HPLC grade. The water used was treated with a Milli-Q water purification system (Millipore, USA). The mobile phase of HPLC gradient was conducted as follows (Table 2). Ginsenosides Rg1, Re, Rb1, Rb2, Rc standards were purchased from BTGin Co., Ltd (Daejeon, Korea). Digoxin was used as internal standard.

Table 2. Mobile phase of HPLC gradient condition for ginsenosides analysis.

Retention time (min)	Solvent (A)	Solvent (B)
0	82	18
22	70	30
32	55	45
50	50	50
55	82	18
60	82	18

(Solvent A: Distilled water, Solvent B: Acetonitrile).

Stock solutions for the 4 ginsenosides were prepared separately in 100% MeOH. Digoxin stock solution was prepared in 70% MeOH. Working solutions were prepared in methanol by mixing known amount of all the ginsenosides together. 5 concentrations were made for standard curves, each concentration was 60, 120, 240, 320, 480 ppm. Ginsenosides were detected at a wavelength of 203 nm with the peak areas corresponding to ginsenosides from the samples matching retention times as authentic ginsenoside standards. Standard was spiked 2 times into sample for determination of each ginsenoside. The recovery test was used to evaluate the accuracy of this quantification method. The average recoveries were determined by the following formula:

$$\text{Recovery (\%)} = (\text{observed amount} - \text{original amount})/\text{spiked amount} \times 100\%$$

$$\text{R.S.D. (\%)} = (\text{S.D.}/\text{mean}) \times 100\%$$

(R.S.D.: Relative Standard Deviation, S.D.: Standard Deviation)

Analysis of ginseng ginsenosides contents were modified according to Son *et al.* (1999a) and Yu *et al.* (2000). The total ginsenoside content was calculated as the sum of individual ginsenoside fractions.

The ginsenoside content of ginseng adventitious roots was calculated as:

$$GC \quad (mg\ g^{-1}) = \frac{SGC \quad (from \quad HPLC) \ (mg\ g^{-1}) \quad x \quad SV\ (l)}{AR\ (g)}$$

(GC: ginsenoside content; SGC: sample ginsenoside concentration from HPLC; SV: sample volume; AR: adventitious root)

The ginsenoside productivity of ginseng adventitious roots was calculated as:

$$GP \quad (mg \quad l^{-1}) = \frac{TGC \ (mg \quad g^{-1}) \quad x \quad HR \quad (g)}{MV \quad (l)}$$

(GP: ginsenoside productivity; TGC: total ginsenoside concentration; HR: harvested root; MV: medium volume)

Statistical Analysis

Statistical analysis was performed according to the SPSS system. Mean and standard errors were used throughout and statiatical significance between the mean values was assessed by applying a Duncan's multiple range test. A probability of $P < 0.05$ was considered significant.

RESULT AND DISCUSSION

Effects of gamma irradiation on adventitious root survival rate

In this experiments, initially we determined the survival rate of adventitious roots at different dosage of γ-irradiation. The growth as well as survival rates of adventitious roots declined with increasing dosage of irradiation (Figure 5). The survival rate of roots completely stopped at 200Gy. The growth of adventitious roots is inhibited over 50Gy treatment and the LD_{50} was established by irradiation of adventitious root by 40Gy based on the survival of main root.

Panax ginseng was irradiated by ^{60}Co gamma ray and the growth of hairy roots was inhibited over 30Gy (Choi *et al*. 2002). As some report, which was established to determine the optimal dose range of gamma source radiation for mutagenic induction on the banana cv. Grand Nain for subsequent mutation breeding in the crop. LD_{50} was established by irradiation of shoot tip explants by 33.6Gy based on the survival of explants and shoot proliferation (Ali 2006).

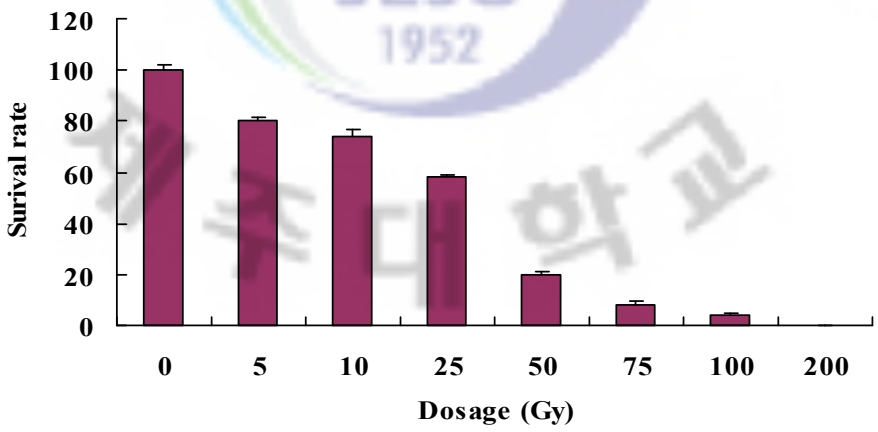


Figure 5. Survival rate among irradiated adventitious roots of *Panax schinseng* Nees with 7 different dosage, growth 5 weeks on petridish containing MS medium with NAA, IAA and 5% sucrose at 23℃ in light condition after irradiation. Bar shown are means ± standard errors of three replicates.

Effects of gamma irradiation on cell line growth

Flask condition After 30 days culture in flask, the values of fresh weight were obtained 3.34 g in control, 2.82 g, 4.57 g, 2.58 g and 3.92 g respectively in cell line 1, 2, 3 and 4 (Figure 6). The values of dry weight were obtained 0.32 g in control, 0.27 g, 0.43 g, 0.25 g and 0.39 g respectively in cell line 1, 2, 3 and 4 (Figure 7). The values of growth ratio were obtained 4.57 in control, 3.91, 6.04, 3.52 and 5.52 respectively in cell line 1, 2, 3 and 4 (Figure 8). The values of growth ratio in cell line 2 and cell line 4 were 32.2% and 20.8% increased compared to the control. The values of growth ratio in cell line 1 and cell line 3 were 14.4% and 22.9% decreased compared to the control.

Bioreactor condition After 30 days culture in bioreactor, the values of fresh weight were obtained 220.5 g in control, 193.8 g, 246.9 g, 140.6 g and 211.9 g respectively in cell line 1, 2, 3 and 4 (Figure 6). The values of dry weight were obtained 19.39 g in control. 17.47 g, 22.64 g, 11.58 g and 19.55 g respectively in cell line 1, 2, 3 and 4 (Figure 7). The values of growth ratio were obtained 14.8 in control, 14.44, 17.15, 8.33 and 14.48 respectively in cell line 1, 2, 3 and 4 (Figure 6). The values of growth ratio in cell line 2 was 15.9% increased compared to the control, but cell line 3 were 43.7% decreased compared to the control, and cell line 1 and 4 were similar with control.

Bioreactor culture system was better to culture adventitious root because of higher growth ratio and more biomass. For *Artemisia annua* transformed hairy root culture, an acoustic mist bioreactor was found to increase root biomass significantly (Chatterjee *et al*. 1997). Adventitious root culture of *Panax ginseng*, was achieved 150-fold growth increases that grown in 500L balloon type bubble bioreactors for 7 weeks (Choi *et al*. 2000). The biomass concentration of *Stizolobium hassjoo* hairy root in the shake flask on the 16th day was 61.2% of that in the mist trickling reactor (Huang et *al*. 2004). *Lithospermum erythrorhizon* S. was irradiated by gamma ray and the cell lines selected in terms of growth rate (similar with control) and appearance of shikonin (Chung *et al.* 2006). In the *Panax*

ginseng hairy roots cultures, for both bioreactors, growth of roots was about three times as high as in the flask cultivation (Jeong *et al*. 2002). The advantageous of theses reactors for the culture of plant cells and organs which are sensitive to shear stress. In this experiment, growth ratio in cell line 2 was increased compared to the control and growth ratio in cell line 1 was similar to the control. An improved method of adventitious root culture system through the use of a bioreactor seems to be a reliable way of commercialization of ginseng.

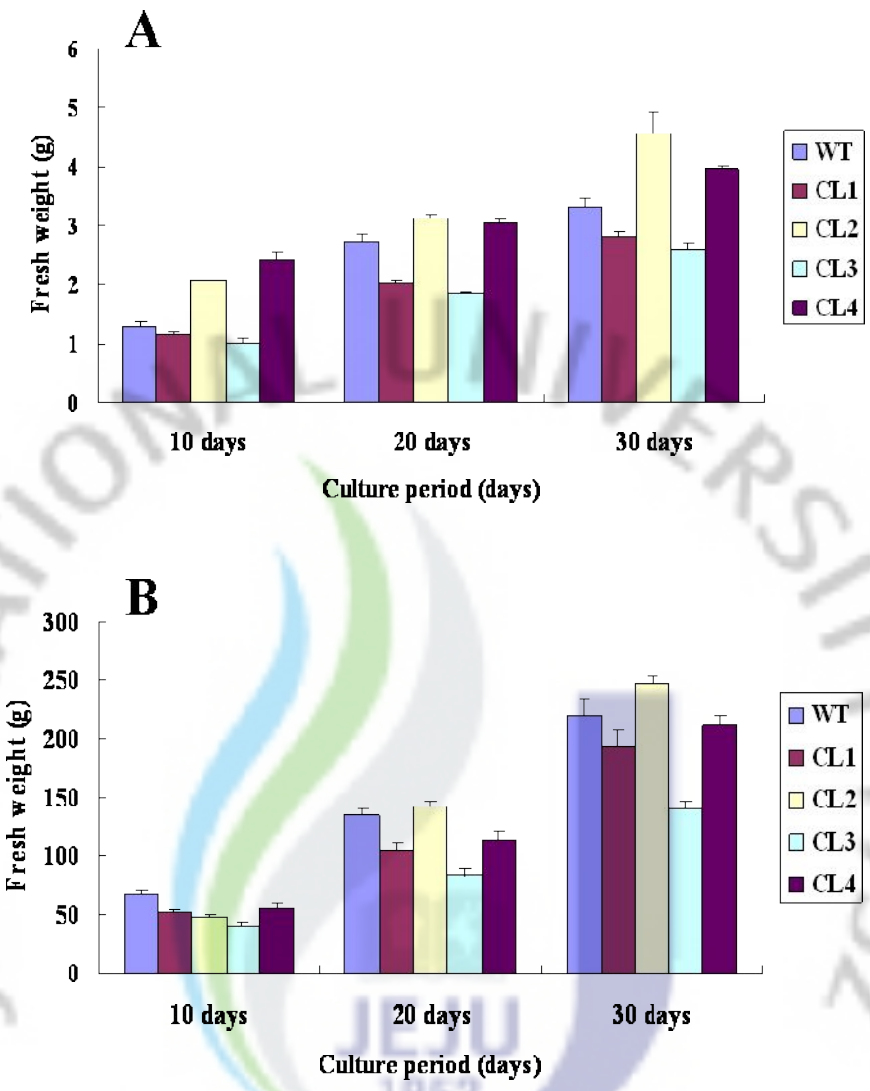


Figure 6. Determinated fresh weight of schinseng adventitious root among control and different cell line after 10, 20 and 30 days of culture in flask containing 50 ml MS medium (A) and in bioreactor with 5-L MS medium (B). The experiments were repeated 3 times.

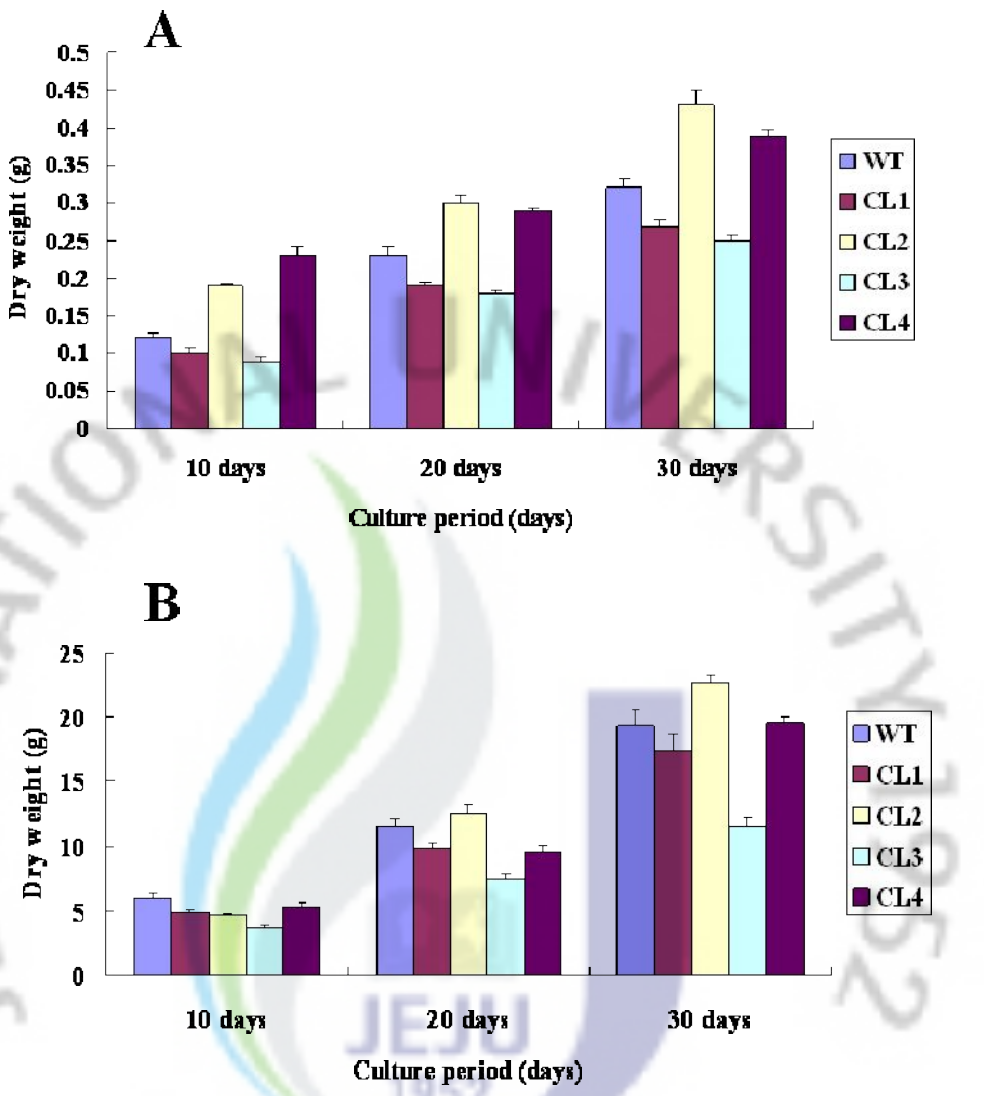


Figure 7. Determinated dry weight of schinseng adventitious root among control and different cell line after 10, 20 and 30 days of culture in flask containing 50 ml MS medium (A) and in bioreactor with 5-L MS medium (B). The experiments were repeated 3 times.

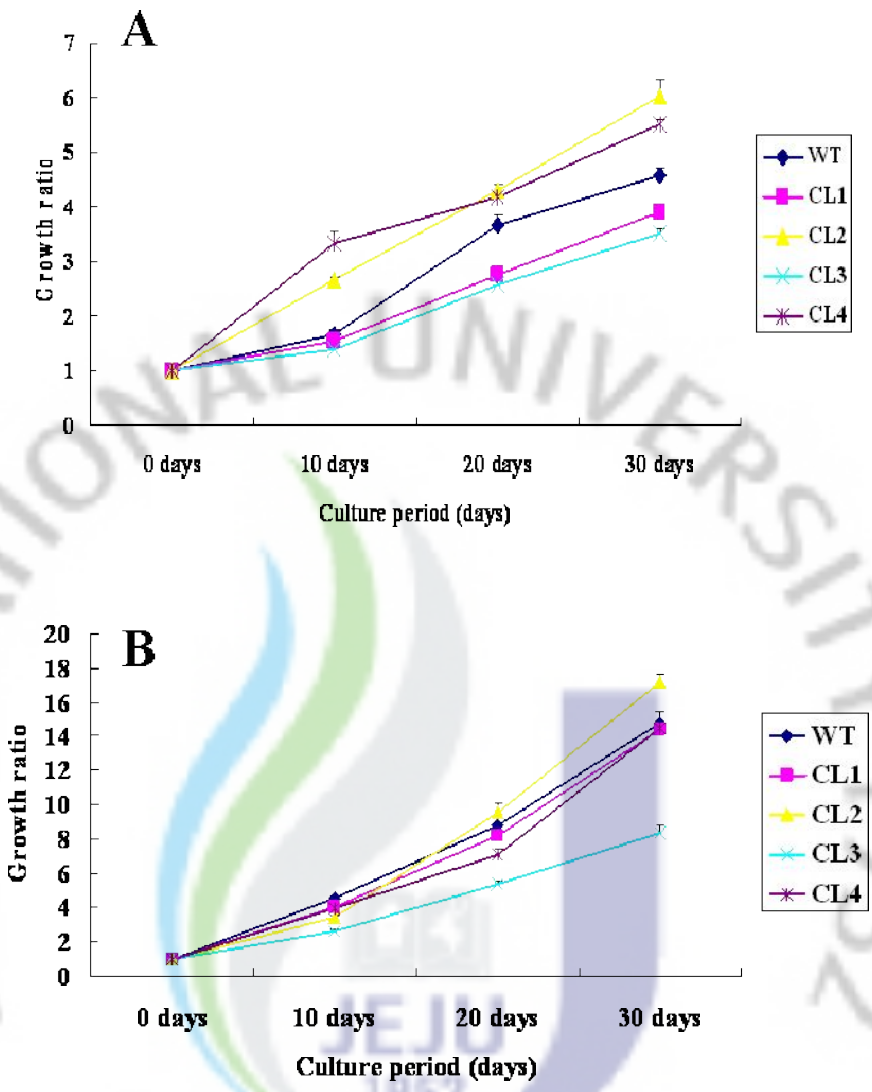


Figure 8. Determinated growth ratio of schinseng adventitious root among control and different cell line after 10, 20 and 30 days of culture in flask containing 50 ml MS medium (A) and in bioreactor with 5-L MS medium (B). The experiments were repeated 3 times.

Morphological characteristics of selected cell line

Phenotype characteristics in flask Secondary root number, length and diameter for the four cell lines of schinseng adventitious root, measured from 40 pieces of main roots (Figure 9 and 10). The values of secondary root number were obtained 372 in control, 244, 408, 286 and 381 respectively in cell line 1, 2, 3 and 4 (Table 3). Cell line 2 was 9.7% increased compared to the control but cell line 1 and 3 was 34.4%, 23.1% decreased compared to the control and cell line 4 were similar with control. The values of secondary root length were obtained 3.51 cm in control, 2.09 cm, 3.22 cm, 2.03 cm and 3.36 cm respectively in cell line 1, 2, 3 and 4 (Table 4). Cell line 1 and 3 was 40.5% and 42.2% decreased compared to the control but cell line 2 and 4 were similar with control. The values of secondary root diameter were obtained 0.31 mm in control, 0.41 mm, 0.36 mm, 0.37 mm and 0.380 mm respectively in cell line 1, 2, 3 and 4 (Table 5) . Cell line 1, 2, 3 and 4 were 31.3%, 15.9%, 20.1% and 21.4% increased compared to the control.

Phenotype characteristics in bioreactor Data presented in (Figure 9 and 10) showed secondary root number, length and diameter for the four cell lines of ginseng adventitious root that measured from 40 pieces of main roots. The values of secondary root number were obtained 1472 in control, 1051, 1965, 962 and 1382 respectively in cell line 1, 2, 3 and 4 (Table 3). Cell line 2 was 33.5% increased compared to the control but cell line 1 and 3 was 28.6%, 34.6% decreased compared to the control and cell line 4 were similar with control. The values of secondary root length were obtained 3.52 cm in control, 2.20 cm, 3.19 cm, 2.07 cm and 3.4 cm respectively in cell line 1, 2, 3 and 4 (Table 4). Cell line 1 and 3 was 36.9% and 41.2% decreased compared to the control but cell line 2 and 4 were similar with control. The values of secondary root diameter were obtained 0.34 mm in control, 0.42 mm, 0.39 mm, 0.38 mm and 0.41 mm respectively in cell line 1, 2, 3 and 4 (Table 5). Cell line 1, 2, 3 and 4 were 22.7%, 14.5%, 11.2% and 22.1% increased compared to the control.

When Grapevine was induced by gamma rays of 2-7Gy (Charbaji *et al*. 1999). Root

length were significantly higher than those of the non-irradiated control at the 2 and 7 Gy dose. Number of leaves of plants was increased when compared with control exposed to 5 and 7Gy. Four Pea (*Pisum sativum* L.) was irradiated by gamma rays of 60-180Gy. Plantlet height and root length were decreased significantly compared to control (ÇİFTÇİ *et al*. 2004). Like our experiment, *Panax schinseng* was irradiated by gamma rays and 4 cell lines were selected from 50-100Gy. Secondary root length and diameter of all 4 cell lines growth in bioreactor were similar with that growth in flask. But secondary root number was higher in bioreactor. In cell line 1, secondary root number and length were decreased compared to the control, but diameter was increased compared to the control. In cell line 2, secondary root number and diameter were increased compared to the control and length was similar to the control. So cell line 1 was negative cell line and cell line 2 was positive cell line. Secondary metabolites of these 2 cell lines maybe increased.

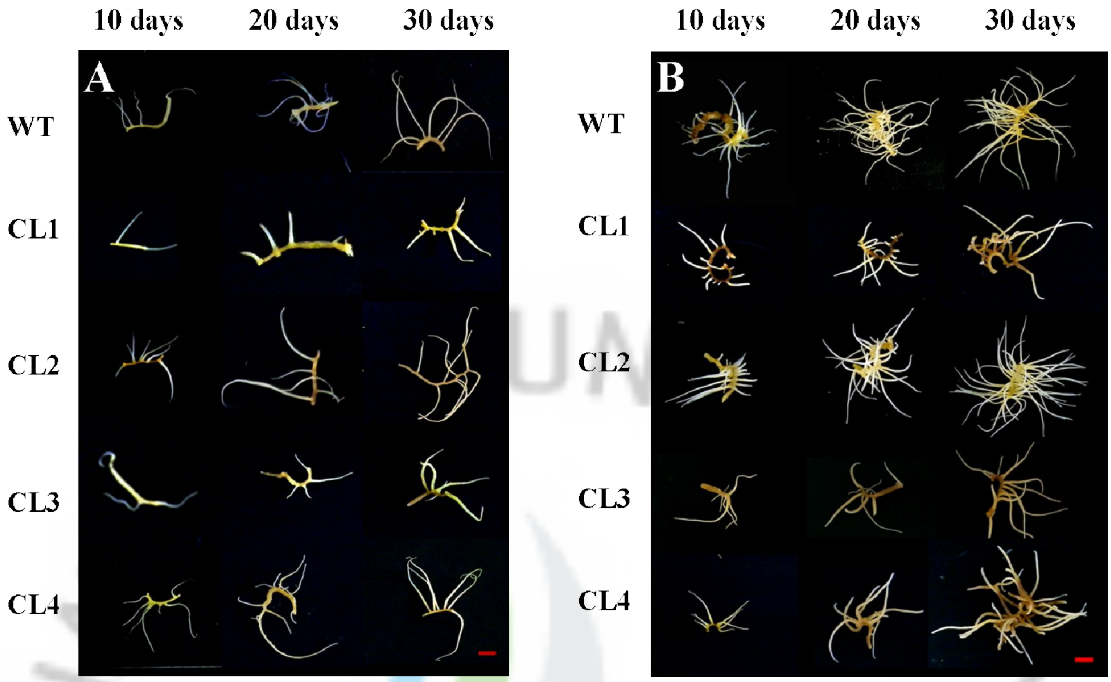


Figure 9. Phenotypic characteristics of secondary roots among control and different cell line growth in flask with 50 ml MS medium (A) and in bioreactor with 5-L MS medium. Bar: 1 cm.

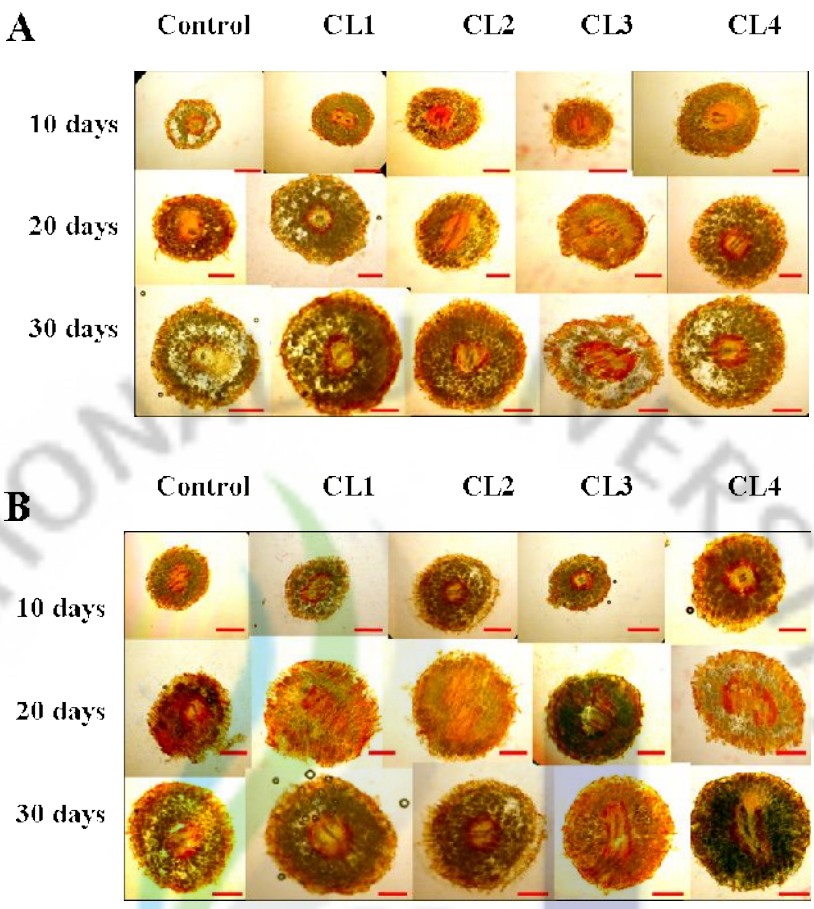


Figure 10. Phenotypic characteristics of secondary root diameter among control and different cell line growth in flask (A) and bioreactor (B). Bar: 0.1 mm

Table 3. Effect of gamma irradiation on secondary root number of schinseng adventitious root among control and different cell line.

Cell Line	Number of secondary root (Flask)			Number of secondary root (Bioreactor)		
	10 days	20 days	30 days	10 days	20 days	30 days
WT	221 b ± 8	314 b ± 6	372 b ± 7	940 a ± 32	1107 b ± 56	1472 b ± 110
CL1	158 d ± 7	185 d ± 10	244 d ± 8	570 b ± 21	683 d ± 27	1051 c ± 56
CL2	292 a ± 5	389 a ± 3	408 a ± 8	545 b c ± 28	1333 a ± 38	1965 a ± 36
CL3	188 c ± 12	254 c ± 17	286 c ± 20	490 c ± 9	671 d ± 27	962 c ± 6
CL4	286 a ± 5	329 b ± 6	381 b ± 4	884 a ± 12	980 c ± 10	1328 b ± 109

Means followed by different letters within a column are significant different at $P < 0.05$ by Duncan's multiple range test. Each treatment was repeated three times.

The date were collected after 30 days of culture in a flask containing 50 ml MS medium and in bioreactor with 5-L MS medium.

Table 4. Effect of gamma irradiation on secondary root length of schinseng adventitious root among control and different cell line.

Cell Line	Length of secondary root (Flask)			Length of secondary root (Bioreactor)		
	10 days	20 days	30 days	10 days	20 days	30 days
WT	1.73 a ± 0.12	2.52 a ± 0.20	3.51 a ± 0.15	1.72 a ± 0.10	2.57 a ± 0.18	3.52 a ± 0.16
CL1	1.17 b ± 0.08	1.81 b ± 0.11	2.09 b ± 0.08	1.15 b ± 0.09	1.87 b ± 0.13	2.20 b ± 0.08
CL2	1.38 b ± 0.06	2.78 a ± 0.10	3.22 a ± 0.08	2.87 a ± 0.15	2.87 a ± 0.15	3.19 a ± 0.18
CL3	0.81 c ± 0.06	1.43 b ± 0.10	2.03 b ± 0.08	1.45 c ± 0.07	1.45 c ± 0.07	2.07 b ± 0.09
CL4	1.89 a ± 0.08	2.61 a ± 0.15	3.36 a ± 0.15	2.80 a ± 0.16	2.80 a ± 0.16	3.4 a ± 0.14

Means followed by different letters within a column are significant different at $P < 0.05$ by Duncan's multiple range test. Each treatment was repeated three times.

The date were collected after 30 days of culture in a flask containing 50 ml MS medium and in bioreactor with 5-L MS medium.

Table 5. Effect of gamma irradiation on secondary root diameter of schinseng adventitious root among control and different cell line.

cell	Diameter of secondary root			Diameter of secondary root		
Line	10 days	20 days	30 days	10 days	20 days	30 days
WT	0.181 c ± 0.013	0.251 d ± 0.012	0.313 c ± 0.011	0.201 b ± 0.019	0.263 b ± 0.016	0.339 b ± 0.018
CL1	0.196 c ± 0.012	0.404 a ± 0.013	0.411 a ± 0.014	0.194 b ± 0.017	0.400 a ± 0.02	0.416 a ± 0.017
CL2	0.274 b ± 0.013	0.329 b c ± 0.015	0.363 b ± 0.013	0.311 a ± 0.016	0.376 a ± 0.016	0.388 a b ± 0.02
CL3	0.129 d ± 0.007	0.299 c ± 0.013	0.376 a b ± 0.009	0.143 c ± 0.013	0.294 b ± 0.016	0.377 a b ± 0.011
CL4	0.309 a ± 0.015	0.342 b ± 0.015	0.380 a b ± 0.017	0.329 a ± 0.019	0.363 a ± 0.02	0.414 a ± 0.02

Means followed by different letters within a column are significant different at $P < 0.05$ by Duncan's multiple range test. Each treatment was repeated three times.

The date were collected after 30 days of culture in a flask containing 50 ml MS medium and in bioreactor with 5-L MS medium.

Effects of gamma irradiation on crude saponin and ginsenoside production

The spectrum of Rg1, Re, Rb1, Rb2 were 202 nm, 198 nm, 196 nm, 200 nm respectively (Figure 12). The overall recoveries ranged from 98 to 113%, with the relative standard deviation ranging from 2.9 to 4.87% (Table 6). These results demonstrated that the HPLC-PDA method is accurate for the quantitative determination of 4 ginsenosides in schinseng samples. Calibration curves of each ginsenoside was made to calculated the content of each ginsenoside (Figure 13).

Flask condition After 30 days of culture in flask, the crude saponin content was higher (9.98%) in cell line 1 compared to other cell lines and control (7.10%). Among the four cell lines, cell line 4 showed a minimum crude saponin content (6.91%) compared to other cell lines and control. In the case of the crude saponin content, cell line 2 (8.81%) and cell line 3 (8.24%) showed higher crude saponin content compared with control (Figure 11). In flask, the chromatogram for 4 major ginsenosides among each cell line and the internal standard is shown in Figure 14, 15, 16, 17. The contents of the 4 kinds of ginsenosides were significantly enriched, especially in cell line 1. The contents of ginsenoside Rg1＋Re, Rb1, Rb2 obtained were 2.31mg g^{-1}, 0.62 mg g^{-1}, 0.21 mg g^{-1} respectively for the control. In cell line 1, levels of ginsenoside Rg1＋Re, Rb1 and Rb2 contents were increased by 1.6-1.8 times that of the control. In cell line 2, levels of ginsenoside Rg1＋Re and Rb1 contents were increased by 1.1-1.2 times that of the control. In cell line 3, levels of each ginsenoside were similar with control. In the case of cell line 4, ginsenoside content was affected negatively in ginseng adventitious root cultures. Overall, the highest ginsenoside yield (29.20 mg g^{-1}) was obtained in cell line 1.

Bioreactor condition After culture in bioreactor, the crude saponin content was higher (13.73%) in cell line 1 compared to other cell lines and control (7.57%). Among the four cell lines, In the case of the crude saponin content, cell line 2 (10.86%), cell line 3 (9.14%) and cell line 4 (9.78%) showed higher saponin content compared with control (Figure 11). To

analyze the amounts of 4 ginsenosides in bioreactor, we used the same HPLC conditions. The chromatogram for 4 major ginsenosides among each cell line is shown in Figure 14, 15, 16, 17. In bioreactor, the contents of ginsenoside Rg1＋Re, Rb1, Rb2 obtained were 2.30 mg g^{-1}, 0.89 mg g^{-1}, 0.25 mg g^{-1} respectively for the control. Levels of ginsenoside Rg1＋Re, Rb1, and Rb2 contents of cell line 1 were increased by 1.8-2.5 times that of the control. Ginsenoside contents of cell line 2 were increased by 1.4-1.5 times that of the control. In cell line 3, levels of Rb2 content were increased by 1.6 times that of the control. In cell line 4, levels of ginsenoside Rg1＋Re content was increased by 1.2 times of the control. Overall, the highest ginsenoside yield (27.06 mg g^{-1}) was obtained in cell line 1.

In conclusion, Crude saponin content in flask was lower than that of bioreactor. ginsenoside content in bioreactor was increased compared with that of flask (Figure 18). Like our result, in the Devil's claw hairy root cultures (*Harpagophytum procumbens*), harpagide content in bioreactor was more than that of flask cultures and had higher levels of sugars and amino acids. Probably due to their better nutrient status and low-stress environments (Ludwig-Műller. *et al*. 2008). Adventitious roots of *Echinacea purpurea* cultivated in 1000-L air lift bioreactors. Dry biomass (5.1 Kg) from adventitious root was achieved and these roots were possessing higher amounts of cichoric acid, chlorogenic acid and caftaric acid (Wu *et al*. 2007). In *Panax ginseng* adventitious root cultures, saponin content in 1000-L bioreactor was about 6 times as much as in small scale culture medium (4, 18, 500-L) (Choi *et al*. 2006). Hairy root culture of *Pueraria phaseoloides*, puerarin content in 2.5-L bioreactor was 200 times as much as in 250 ml flask cultures (Kintzios *et al*. 2004). A mist reactor and a bubble column reactor were used for *Artemisia annua* hairy root culture. Mist reactors produce significantly more artemisinin, while bubble column reactors produce greater biomass. The roots grown in shake flasks contain a negligible amount of artemisinin (Souret *et al*. 2001). As our result, crude saponin content of cell line 1 in bioreactor was increased by 2.2 folds compared with that of flask. Ginsenoside content was increased by 2.1 folds that of the flask. Crude saponin content of cell line 2 was increased by 1.43 folds that of flask. Ginsenoside content was increased by 1.38 folds that of the flask. Cell line 1 was

the best cell line because of the highest crude saponin and ginsenoside content in bioreactor.

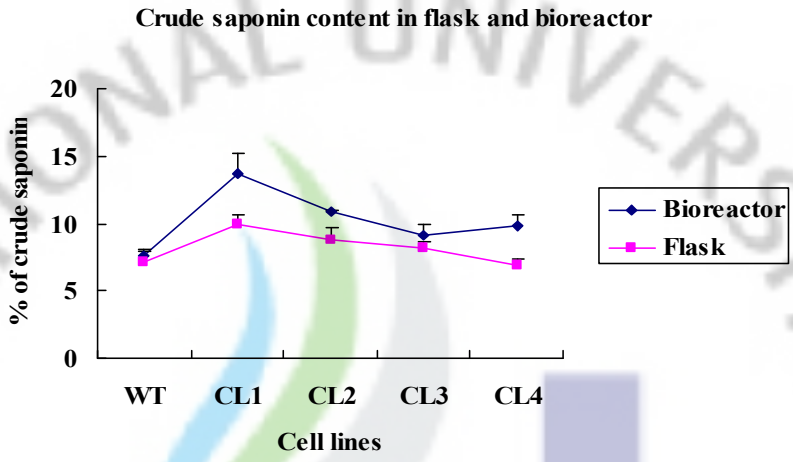


Figure 11. Effect of γ-ray on crude saponin content among control and different cell lines after 30 days growth in flask with 50 ml MS medium (■) and in bioreactor with 5-L MS medium (◆). Bar shown are means ± standard errors of three replicates.

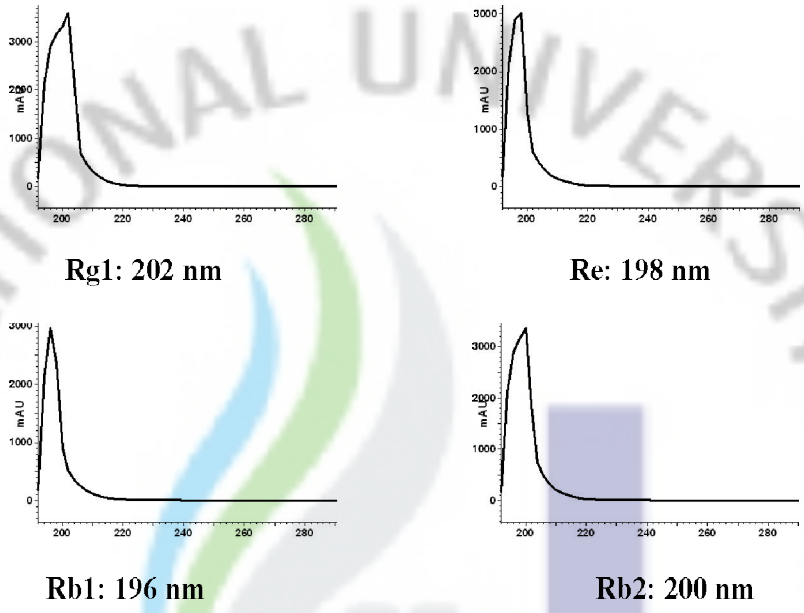


Figure 12. Typical UV absorption spectrum of ginsenoside Rg1, Re, Rb1 and Rb2.

Table 6. Spiked method for the determination of 4 ginsenosides.

Compound (mg)	Original (mg)	Spiked (mg)	Observed (%)	Recovery (%)	Mean (%)	R.S.D. (%)
Rg1	0.65	0.15	0.82	113	110.5	3.19
		0.25	0.92	108		
Re	2.03	0.1	2.11	98	101.5	4.87
		0.2	2.24	105		
Rb1	0.81	0.1	0.92	110	107.5	3.28
		0.2	1.02	105		
Rb2	2.54	0.5	3.04	100	102.1	2.9
		0.7	3.27	104.2		

Recovery (%) = (observed amount−original amount)/spiked amount×100%;

R.S.D. (%) = (S.D./mean)×100%.

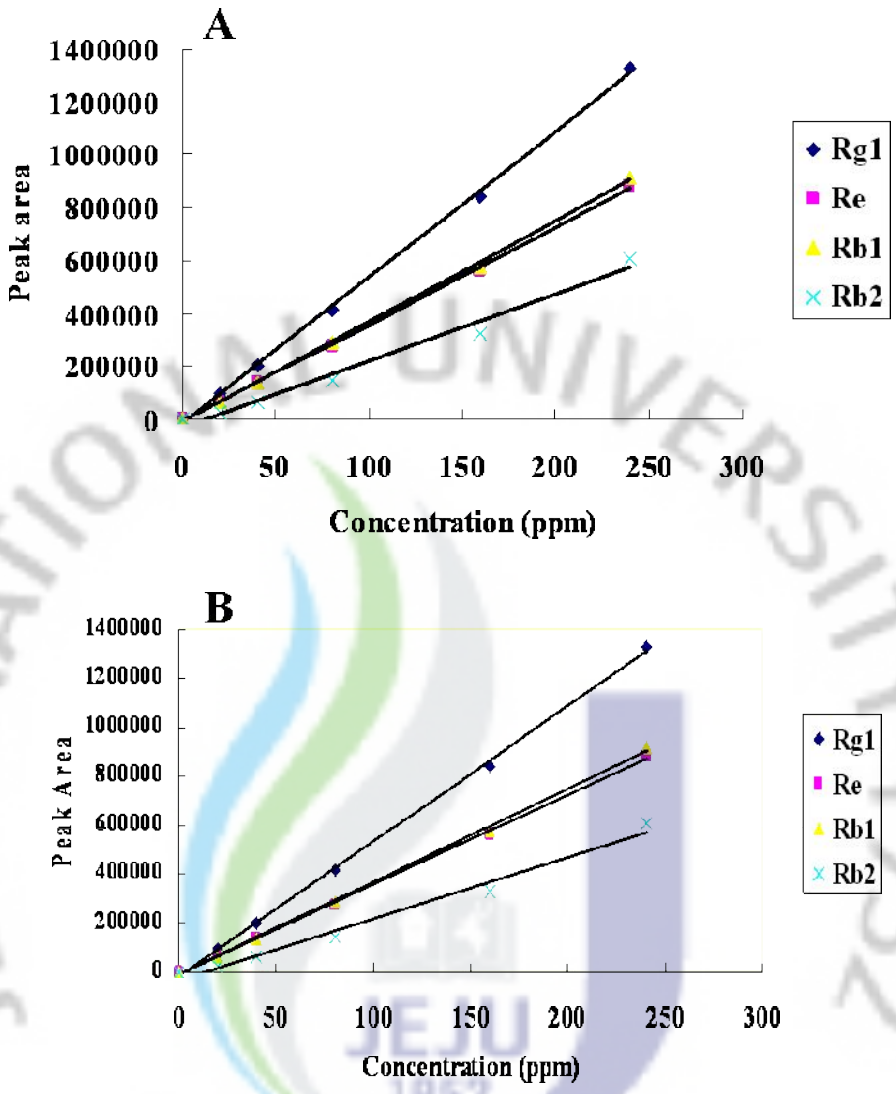


Figure 13. Standard curves of ginsenoside Rg1, Re, Rb1, Rb2 for ginsenoside calculation in flask (A) and bioreactor (B).

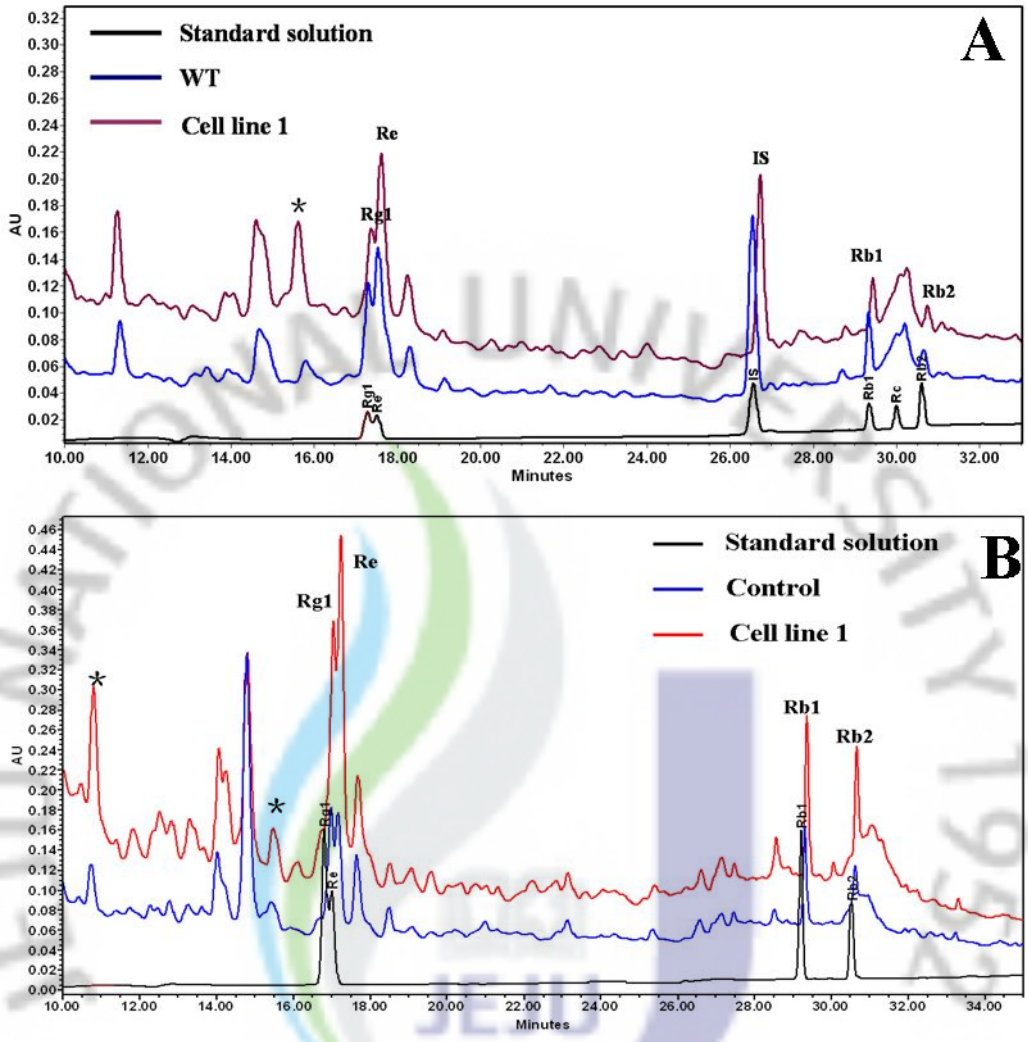


Figure 14. Typical chromatograms obtained from standard solution, control and cell line 1 solution extracted from flask (A) and bioreactor (B) by UV detection at 203 nm.

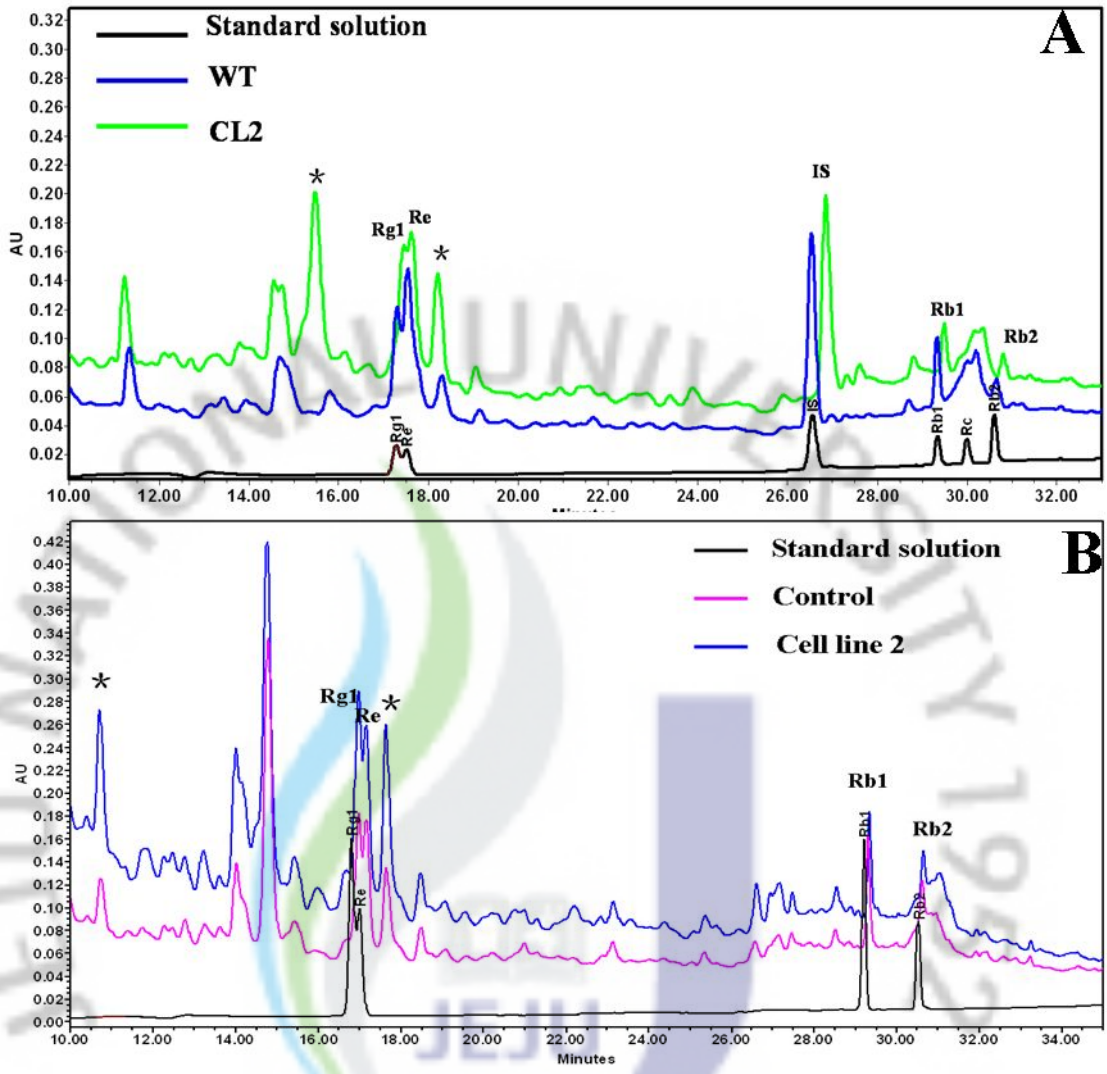


Figure 15. Typical chromatograms obtained from standard solution, control and cell line 2 solution extracted from flask (A) and bioreactor (B) by UV detection at 203 nm.

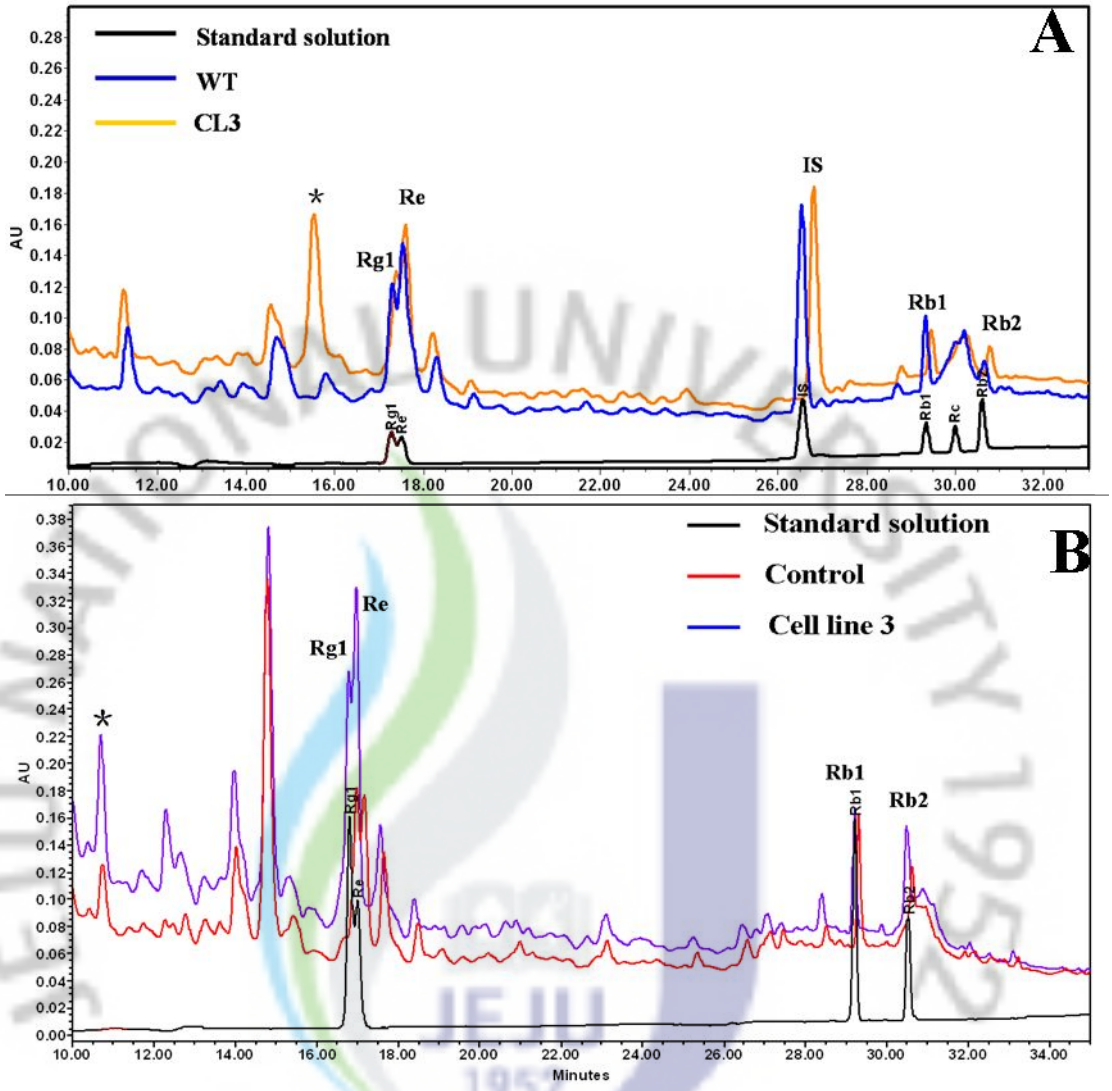


Figure 16. Typical chromatograms obtained from standard solution, control and cell line 3 solution extracted from flask (A) and bioreactor (B) by UV detection at 203 nm.

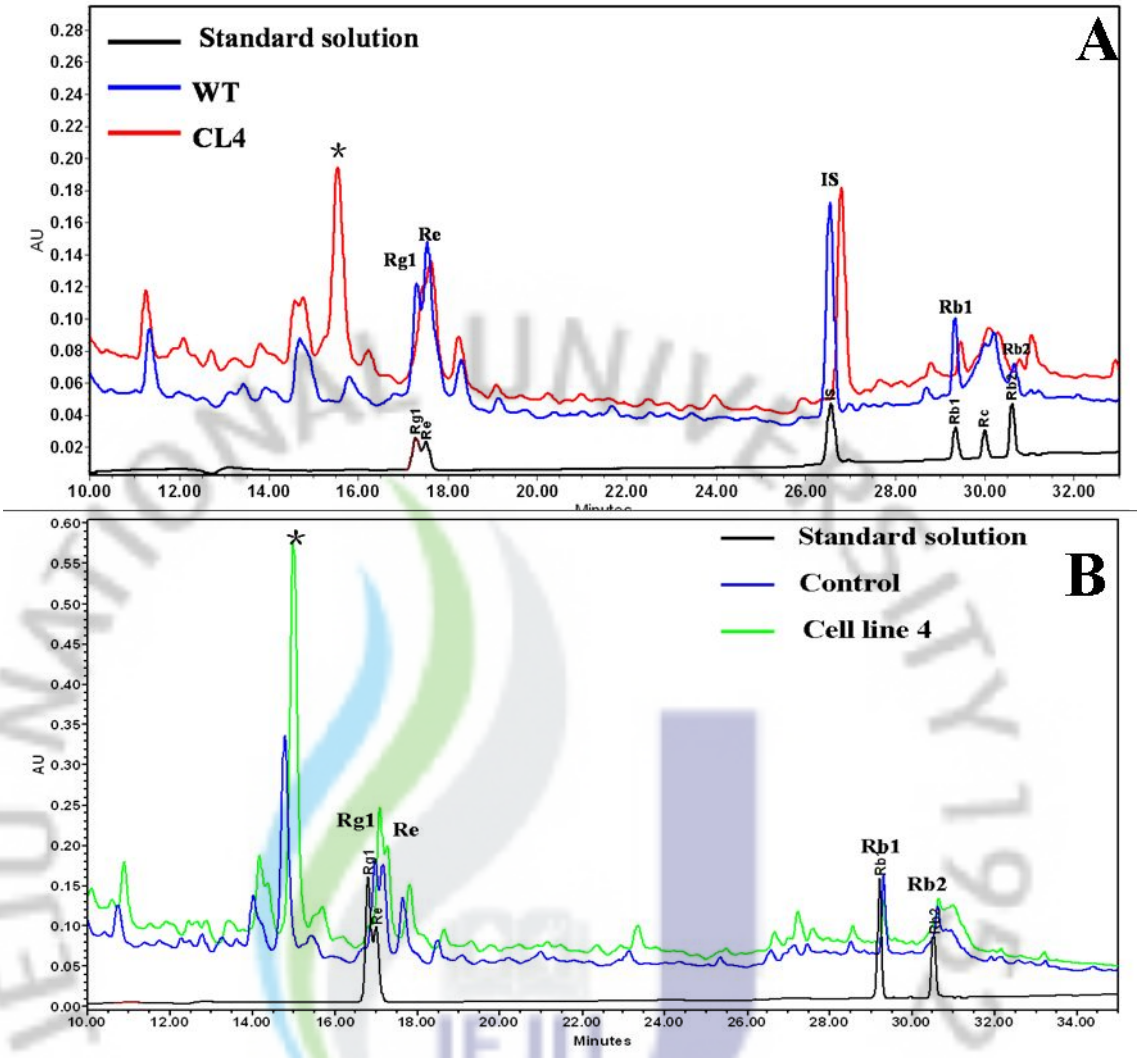


Figure 17. Typical chromatograms obtained from standard solution, control and cell line 4 solution extracted from flask (A) and bioreactor (B) by UV detection at 203 nm.

Table 7. Ginsenoside production of *Panax schinseng* among control and different cell line. The date were collected after 30 days of culture in a 100 ml Erlenmeyer flask containing 50 ml MS medium.

Cell line	Ginsenoside (mg g^{-1}DW)				Ginsenoside productivity (mg l^{-1})
	Rg1+Re	Rb1	Rb2	4 ginsneosides contents	
WT	2.31 b±0.11[a)] (100)[b)]	0.62 b c ± 0.03 (100)	0.21 a ± 0.03 (100)	3.14 b ± 0.37 (100)	20.04 b ± 2.28 (100)
CL1	4.07 a±0.24 (176)	0.99 a ± 0.11 (160)	0.35 a ± 0.01 (167)	5.41 a ± 0.19 (159)	29.20 a ± 1.25 (146)
CL2	2.43 b±0.13 (105)	0.71 b ± 0.10 (115)	0.20 a ± 0.05 (95.2)	3.34 b ± 0.07 (106)	28.48 a ± 1.22 (142)
CL3	2.28 b±0.21 (98.7)	0.47 c d ± 0.04 (75.8)	0.20 a ± 0.02 (95.2)	2.96 b c ± 0.27 (94.3)	14.67 b ± 1.77 (73.2)
CL4	1.72 b c±0.12 (74.5)	0.36 d ± 0.02 (58.1)	0.16 a ± 0.04 (76.2)	2.20 d ± 0.33 (70.1)	17.04 b ± 2.52 (85)

a) Means followed by different letters within a column are significant different at $P < 0.05$ by Duncan's multiple range test. Each treatment was repeated three times.
The date were collected after 30 days of culture in a 100 ml Erlenmeyer flask containing 50 ml MS medium.

b) Percentage of response $= \frac{\text{Treatment}}{\text{WT}} \text{X } 100$

Table 8. Ginsenoside production of *Panax schinseng* among control and different cell line. The date were collected after 30 days of culture in a 15L bioreactor containing 5L MS medium.

Cell line	Ginsenoside (mg g^{-1}DW)				Ginsenoside productivity (mg l^{-1})
	Rg1+Re	Rb1	Rb2	4 ginsenosides contents	
WT	2.30 c±0.26[a)] (100)[b)]	0.89 b ± 0.19 (100)	0.25 b ± 0.02 (100)	3.44 c ± 0.38 (100)	13.21 c± 1.11 (100)
CL1	5.64 a±0.06 (245)	1.62 a ± 0.11 (182)	0.49 a ± 0.01 (196)	7.76 a ± 0.12 (226)	27.06 a ± 1.47 (205)
CL2	3.39 b±0.13 (147)	0.91 b ± 0.03 (102.3)	0.34 b ± 0.05 (136)	4.64 b ± 0.13 (135)	20.98 b ± 0.4 (159)
CL3	2.39 c±0.19 (104)	0.59 b ± 0.02 (66.3)	0.39 a b ± 0.05 (156)	3.37 c ± 0.15 (98)	7.79 d ± 0.5 (59)
CL4	2.85 b c±0.28 (124)	0.77 b ± 0.06 (86.5)	0.2 b ± 0.06 (80)	3.82 b c ± 0.36 (111)	15.79 b ± 1.84 (120)

a) Means followed by different letters within a column are significant different at $P < 0.05$ by Duncan's multiple range test. Each treatment was repeated three times.
The date were collected after 30 days of culture in a 15-L bioreactor containing 5-L MS medium.

b) $\text{Percentage of response} = \frac{\text{Treatment}}{\text{WT}} \text{X } 100$

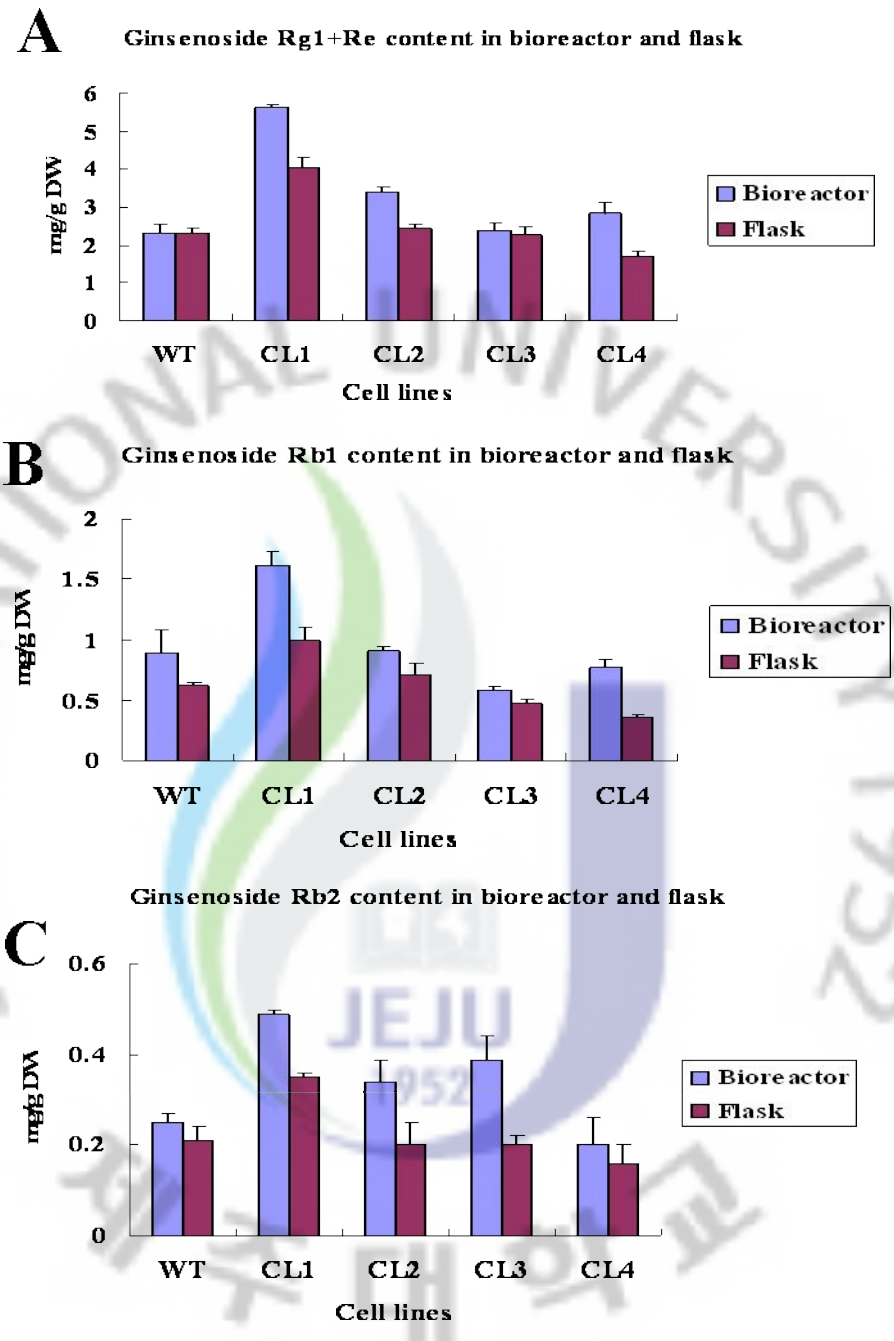


Figure 18. Compared ginsenoside Rg1+Re (A), Rb1 (B), Rb2 (C) contents in flask and bioreactor.

TOTAL DISSCUSSION

There are two major groups of ginsenosides Rb and Rg characterized from the *Panax* species and they have 20 (S) protopanaxadiols and 20 (S) protopanaxatriols respectively. The Rb group includes the ginsenosides Rb1, Rb2, Rc and Rd, while Rg group includes the Re, Rf and Rg1 (Tanaka and Kasai, 1984). For our present investigation, depending on the wide usage of ginsenosides, ginsenosides of Rg1, Re, Rb1 and Rb2 were selected for characterization from *Panax schinseng* root cultures after irradiation. Some method to determination ginseng quality by using Rg1, Re and Rb1 (Wu *et al*. 2007). Some ginsenosides, like Rg3, Rh2 and Rh1 were difficult to be detected in cultured ginseng. Ginsenoside Rb1, Re, Rg1 and Rb2 were the precursors to form other ginsenosides by Human Intestinal Microflora. For example, PD (panaxadiol) ginsenosides Rb1, Rb2, and Rc of fresh and white ginsengs are transformed to 20-*O*-*β*-D-glucopyranosyl-20(*S*)-PD (compound K) by human intestinal bacteria (Akao *et al*. 1998b, Bae *et al*. 2002a, Bae *et al*. 2000). The PD ginsenosides Rg3 and Rg5 of red ginseng are transformed to ginsenosides Rh2 and Rh3, respectively (Bae *et al*. 2002b, Bae *et al*. 2004a). The PT (panaxatriol) ginsenosides Re and Rg1 are transformed to ginsenoside Rh1 and further to PT (Figure 19) (Bae *et al*. 2005, Wang *et al*. 2000).

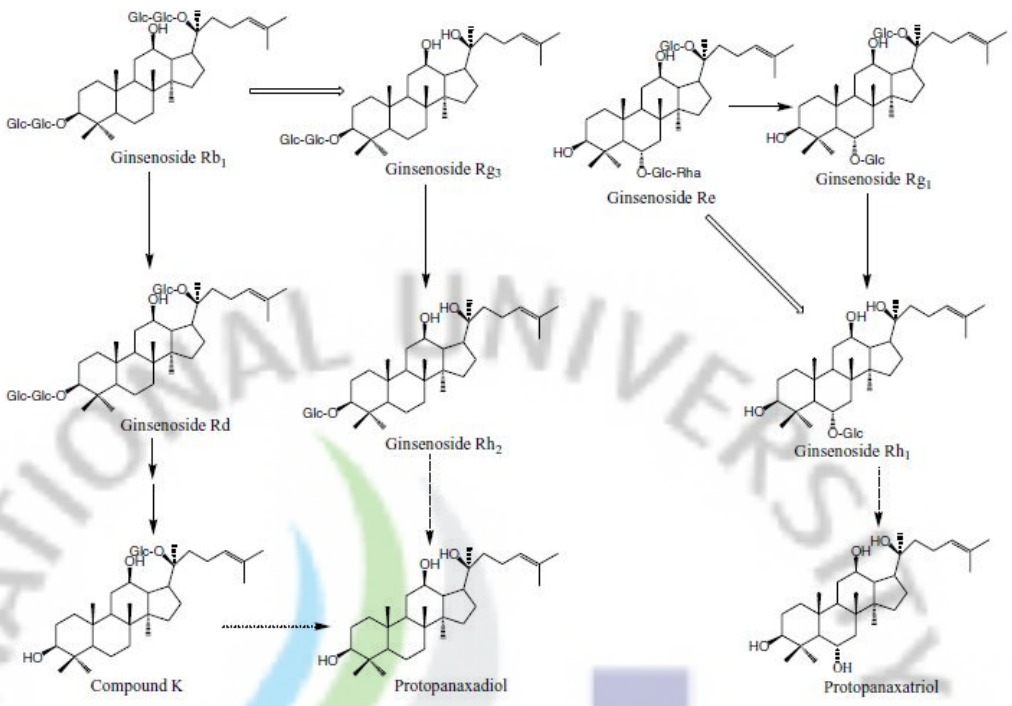


Figure 19. Structures and metabolic pathways of ginsenoside Rb1, Re, Rg1, and Rg3 by human intestinal microflora (→, main pathway by intestinal microflora; ·····>, minor pathway by intestinal microflora; ⟹, chemical transformation by steaming).

Like some report (Charbaji et al. 1999, ÇİFTÇİ *et al*. 2004), different morphological characteristics were induced by gamma irradiation among different plant. In this experiment, secondary root number, length, diameter of *Panax schinseng* in flask and bioreactor induced by gamma irradiation had different morphologies. In cell line 1, secondary root number and length were decreased compared to the control, but diameter was increased compared to the control. In cell line 2, secondary root number and diameter were increased compared to the control and length was similar to the control.

As it is known, in root cultures, the ginsenoside yield depends not only on the accumulation of the commercially useful compounds but also on their biomass production capability. We selected the best cell line was cell line 1 in this experiment. Because both in flask and bioreactor, each ginsenoside content was highest compared to control and other cell

lines. Bioreactor culture system was better to culture adventitious root, because of higher growth ratio and more biomass. Some ginsenosides contents in bioreactors were also increased compared with that of flasks. A few types of bioreactors are commonly used for plant cell suspension cultures such as the mechanically-agitated (stirred-tank), airlift, bubble column, liquid-dispersed bioreactor. We use bubble column bioreactor for *Panax schinseng* adventitious roots culture, because the bubble column reactor is one of simplest types of reactors and is easy to scale-up. Like an airlift bioreactor, the bubbles in a bubble column create less shear stress compared to other stirred types, so that it is useful for organized structures such as adventitious roots (Choi *et al*. 2006).

Gamma irradiation is powerful and useful tool for the enhancement of production of secondary metabolites and for the bioreactor culture system. This result maybe due to an alteration in the biosynthetic activity of the ginsenosides induced by the gamma irradiation. According to the result, highest ginsenoside content was determinated in cell line 1. So cell line 1 was the best cell line. This cell line will be also useful to research key genes related to the biosynthetic pathway of the ginsenosides in *Panax schinseng*.

REFERENCE

Abe I, Rohmer M, Orestwich GD. 1993. Enzymatic cyslization of squalene and oxidosqualene to sterols and triterpene. *Chemical Reviews* 93: 2189-206.

ATAK M, ÖZCAN S. 2006. Use of Gamma Rays to Induce Mutations in Four Pea (*Pisum sativum* L.) Cultivars. *Turk Journal of Biology* 30: 29-37.

Ali M-A. 2006. Optimal dose rate of gamma irradiation and EMS concentration for mutation induction on shoot tip of Banana cv. Grand Nain. *Agricultural Research and Technology Corporation Unit.*

Asaka I, Ii I, Hirotani M, Asada Y, Furuya T. 1993. Production of ginsenoside saponins by culturing ginseng (*Panax ginseng*) embryogenic tissues in bioreactors. *Biotechnology Letters* 15: 1259-64.

Akao T, Kida H, Kanaoka M, Hattori M, Kobashi K. 1998b. Intestinal bacterial hydrolysis is required for the appearance of compound K in rat plasma after oral administration of ginsenoside Rb1 from *Panax ginseng*. *Journal of Pharmacy and Pharmacology* 50: 1155-1160.

Bae T-W, Kim J, Song I-J, Song S-Y, Lim P-O, et al. 2009. Production of unbolting lines through gamma-ray irradiation mutagenesis in genetically modified herbicide-tolerant Zoysia japonica. *Breeding Science* 59: 103-5.

Bae EA, Choo MK, Park EK, Park SY, Shin HY, Kim DH. 2002a. Metabolism of ginsenoside Rc by human intestinal bacteria and its related antiallergic activity. *Biological & Pharmaceutical Bulletin* 25: 743-747.

Basar-Eroglu C, Brand A, Hildebrandt H, Karolina Kedzior K, Mathes B, Schmiedt C. 2007. Working memory related gamma oscillations in schizophrenia patients. *International Journal of Psychophysiology* 64: 39-45.

B. Senthamizh Selvi B, Ponnuswami V, Sumathi T. 2007. Identification of DNA Polymorphism induced by Gamma Ray Irradiation in Amla (Emblica Officinalis Gaertn.) Grafts of V1 M1 and V2 M1 Generation. *Journal of Applied Sciences Research*, 3(12): 1933-1935.

Bae EA, Shin JE, Kim DH. 2005. Metabolism of ginsenoside Re by human intestinal microflora and its estrogenic effect. *Biological & Pharmaceutical Bulletin* 28: 1903-8.

Bae EA, Park SY, Kim DH. 2000. Constitutive beta-glucosidases hydrolyzing ginsenoside Rb1 and Rb2 from human intestinal bacteria. *Biological & Pharmaceutical Bulletin* 23: 1481-1485.

Bae EA, Han MJ, Choo MK, Park SY, Kim DH. 2002b. Metabolism of 20(S) - and 20(R)-ginsenoside Rg3 by human intestinal bacteria and its relation to in vitro biological activities. *Biological & Pharmaceutical Bulletin* 25: 58-63.

Bae EA, Han MJ, Kim EJ, Kim DH. 2004a. Transformation of ginseng saponins to ginsenoside Rh2 by acids and human intestinal bacteria and biological activities of their transformants. *Pharmaceutical Society of Korea* 27: 61-67.

Bae KH, Choi YE, Shin CG, Kim YY, Kim YS. 2006. Enhanced ginsenoside productivity by combination of ethephon and methyl jasmonate in ginseng (*Panax ginseng* C.A. Meyer) adventitious root cultures. *Biotechnology Letters* 28: 1163–1166.

Chung BY, Lee YB, Baek MH, Kim JH, Wi SG, Kim JS. 2006. Effects of low-dose gamma-irradiation on production of shikonin derivatives in callus cultures of Lithospermum erythrorhizon S. *Radiation Physics and Chemistry* 75: 1018-23.

Chang, H., & But, P. (Eds.). 1986. Pharmacology and applications of Chinese materia medica: *World Scientific Publishing Co., Inc.*

Chatterjee, C., M. J. Corell, P. J. Weathers, B. E. Wyslouzil, and D. B. Wacers. 1997. Simplified acoustic window mist bioreactor. *Biotechnology Techniques* 11:155–158.

Choi S. 2002. Epidermis proliferative effect of the Panax ginseng ginsenoside Rb 2. *Archives of Pharmacal Research* 25: 71-6.

Choi D-W, Jung J, Ha YI, Park H-W, In DS, et al. 2005. Analysis of transcripts in methyl jasmonate-treated ginseng hairy roots to identify genes involved in the biosynthesis of ginsenosides and other secondary metabolites. *Plant Cell Reports* 23: 557-66.

Choi K-M, Kwon J-H, Sung-Hee Ban S-H, Yang D-C. 2002. Production of Ginsenoside in the Hairy Roots Irradiated by 60Co γ-ray on *Panax ginseng* C.A Meyer. *Journal of Ginseng Research.*Vol. 26, No. 4, 219-225.

CHOI Y-E, KIM Y-S, PAEK K-Y. 2006. TYPES AND DESIGNS OF BIOREACTORS FOR HAIRY ROOT CULTURE. *S. Dutta Gupta and Y. Ibaraki (eds.), Plant Tissue Culture Engineering*, 161–172.

Chan RY, Chen WF, Dong A, Guo D, Wong MS. 2002. Estrogen-like activity of ginsenoside Rg1 derived from *Panax notoginseng*. *Journal of Clinical Endocrinology & Metabolism*. 87: 3691-5.

Chen JC, Chen LD, Tsauer W, Tsai CC, Chen BC, Chen YJ. 2001. Effects of Ginsenoside Rb2 and Rc on inferior human sperm motility in vitro. *American Journal of Chinese Medicine*. 29: 155-60.

Charbaji T, Nabulsi I. 1999. Effect of low doses of gamma irradiation on in vitro growth of grapevine. *Plant Cell, Tissue and Organ Culture* 57: 129-32.

Chang T-L, Ding H-Y, Kao Y-W. 2008. Role of Ginsenoside Rd in Inhibiting 26S Proteasome Activity. J*ournal of Agricultural and Food Chemistry* 56: 12011-5.

Gyawali R, Seo H-Y, Lee H-J, Song H-P, Kim D-H, et al. 2006. Effect of γ-irradiation on volatile compounds of dried Welsh onion (Allium fistulosum L.). *Radiation Physics and Chemistry* 75: 322-8.

Hobbs C. 1996. Ginseng: The Energy Herb, Botanica Press, Loveland, Connecticut, USA.

Han J, Choi Y. 2009. Rapid induction of Agrobacterium tumefaciens-mediated transgenic roots directly from adventitious roots in Panax ginseng. *Plant Cell, Tissue and Organ Culture* 96: 143-9.

Hady M-S Abdel, Ali Z-A. 2006. Effect of Gamma Irradiation on Wheat Immature Culture Regenerated Plants. *Journal of Applied Sciences Research* 2(6): 310-316.

Hu F-X, Zhong J-J. 2008. Jasmonic acid mediates gene transcription of ginsenoside

biosynthesis in cell cultures of Panax notoginseng treated with chemically synthesized 2-hydroxyethyl jasmonate. *Process Biochemistry* 43: 113-8.

Harrison R-G. 1990. Hybrids and hybrid zones: historical perspective. Published by Oxford University Press. Inc.

Ingram B, Mavituna E. 2000. Effect of bioreactor configuration on the growth and maturation of *Picea sitchensis* somatic embryo cultures. *Plant Cell, Tissue and Organ Culture* 61: 87–96, 2000.

Jeong C, Hyun J, Kim Y, Lee E-S. 2003. Ginsenoside RB1 the anti-ulcer constituent from the head of Panax ginseng. *Archives of Pharmacal Research* 26: 906-11.

Jung JD, Park HW, Hahn Y, Hur CG, In DS, et al. 2003. Discovery of genes for ginsenoside biosynthesis by analysis of ginseng expressed sequence tags. *Plant Cell Reports* 22: 224-30.

Joseph R, Yeoh H-H, Loh C-S. 2004. Induced mutations in cassava using somatic embryos and the identification of mutant plants with altered starch yield and composition. *Plant Cell Report.* 23: 91–98.

Jeong G, Park D, Hwang B, Park K, Kim S, Woo J. 2002. Studies on mass production of transformed Panax ginseng hairy roots in bioreactor. *Applied biochemistry and biotechnology* 98: 1115-27.

Kim O, Bang K, Kim Y, Hyun D, Kim M, Cha S. 2009. Upregulation of ginsenoside and gene expression related to triterpene biosynthesis in ginseng hairy root cultures elicited by methyl jasmonate. *Plant Cell, Tissue and Organ Culture* 98: 25-33.

Kim Y, Hahn E, Murthy H, Paek K. 2005. Adventitious root growth and ginsenoside accumulation in *Panax ginseng* cultures as affected by methyl jasmonate. *Biotechnology Letters* 26: 1619-22.

Kwon J-H, Bélanger JMR, Paré JRJ, Yaylayan VA. 2003. Application of the microwave-assisted process (MAP$^{TM☆}$) to the fast extraction of ginseng saponins. *Food Research International* 36: 491-8.

Kim O, Bang K, Kim Y, Hyun D, Kim M, Cha S. 2009. Upregulation of ginsenoside and gene expression related to triterpene biosynthesis in ginseng hairy root cultures elicited by methyl jasmonate. *Plant Cell, Tissue and Organ Culture* 98: 25-33.

Kiong A, Lai A, Hussein S, Harun A. 2008. Physiological Responses of Orthosiphon stamineus Plantles to Gamma Irradiation. *American-Eurasian Journal of Sustainable Agriculture* 2: 135-49.

Kang K-S, Kang B-C, Lee B-J, Che J-H, Li G-X, et al. 2000. Preventive effect of epicatechin and ginsenoside Rb2 on the inhibition of gap junctional intercellular communication by TPA and H_2O_2. *Cancer Letters* 152: 97-106.

Ketchum R-E-B, Gibson D-M, Croteau R-B, Shuler M-L. 1999. The kinetics of taxoid accumulation in cell suspension cultures of Taxus following elicitation with methyl jasmonate. *Biotechnology & Bioengineering* 62: 97±105.

Kintzios S, Makri O, Pistola E, Matakiadis T, Ping Shi H, Economou A. 2004. Scale-up production of puerarin from hairy roots of *Pueraria phaseoloides* in an airlift bioreactor. *Biotechnology Letters* 26: 1057-9.

Lee MH, Jeong JH, Seo JW, Shin CG, Kim YS, et al. 2004. Enhanced triterpene and phytosterol biosynthesis in *Panax ginseng* overexpressing squalene synthase gene. *Plant Cell Physiology* 45: 976-84.

Lee Y, Jin Y, Lim W, Ji S, Cho J, et al. 2003. Ginsenoside Rc and Re stimulate c-Fos expression in MCF-7 human breast carcinoma cells. *Archives of Pharmacal Research* 26: 53-7.

Lee Y, Jin Y, Lim W, Ji S, Choi S, et al. 2003. A ginsenoside-Rh1, a component of ginseng saponin, activates estrogen receptor in human breast carcinoma MCF-7 cells. *The Journal of Steroid Biochemistry and Molecular Biology* 84: 463-8.

Ludwig-Müller J, Georgiev M, Bley T. 2008. Metabolite and hormonal status of hairy root cultures of Devil's claw (*Harpagophytum procumbens*) in flasks and in a bubble column bioreactor. *Process Biochemistry* 43: 15-23.

Lu MB, Wong HL, Teng WL. 2001. Effects of elicitation on the production of saponin in cell culture of *Panax ginseng*. *Plant Cell Report* 20: 647–677.

Mabberley. D-J. 1987. The Plant-Book. Cambridge Univ. Press, Cambridge.

Mallol A, Cusidó RM, Palazón J, Bonfill M, Morales C, Piñol MT. 2001. Ginsenoside production in different phenotypes of *Panax ginseng* transformed roots. *Phytochemistry* 57: 365-71.

Mokobia C, Okpakorese E-M, Analogbei C, Agbonwanegbe J. Effect of gamma irradiation on the grain yield of Nigerian *Zea mays* and *Arachis hypogaea*. *Journal of Radiological Protection* 26: 423–427.

Ngan F, Shaw P, But P, Wang J. 1999. Molecular authentication of Panax species. *Phytochemistry* 50: 787-91.

Nah S-Y, Kim D-H, Rhim H. 2007. Ginsenosides: Are Any of them Candidates for Drugs Acting on the Central Nervous System? *CNS Drug Reviews* 13: 381-404.

Nakata H, Kikuchi Y, Tode T, Hirata J, Kita T, et al. 1998. Inhibitory Effects of Ginsenoside Rh2 on Tumor Growth in Nude Mice Bearing Human Ovarian Cancer Cells. *Cancer Science* 89: 733-40.

Nah S-Y.1997. Ginseng: Recent advances and Trends. *Journal of Ginseng Research* 21:1-12.

Park J-Y, C. Y. Lee C-Y, Won J-Y. 2007. Analytical optimum of ginsenosides according to the gradient elution of mobile phase in high performance liquid chromatography. *Korean J. Medicinal Crop Science.* 15(3): 215-219.

Park E-K, Choo M-K, Kim E-J, Han MJ, Kim D-H. 2003. Antiallergic Activity of Ginsenoside Rh2. *Biological & Pharmaceutical Bulletin* 26: 1581-4.

Park E-K, Choo M-K, Oh JK, Ryu JH, Kim D-H. 2004. Ginsenoside Rh2 Reduces Ischemic Brain Injury in Rats. *Biological & Pharmaceutical Bulletin* 27: 433-6.

Park YC, Lee CH, Kang HS, Kim K-W, Chung HT, Kim HD. 1996. Ginsenoside-Rh1 and Rh2 inhibit the induction of nitric oxide synthesis in murine peritoneal macrophages. *Biochemistry and Molecular Biology International* 40: 751 – 7.

Park E, Choo M, Han M, Kim D. 2000. Ginsenoside Rh1 possesses antiallergic and anti-inflammatory activities. *International archives of allergy and immunology* 133: 113-20.

Pauwels L, Morreel K, Witte ED, Lammertyn F, Montagu MV, Boerjan W, Inz´e D, Goossens A. 2008. Mapping methyl jasmonate-mediated transcriptional reprogramming of metabolism and cell cycle progression in cultured Arabidopsis cells. *Proceedings of the National Academy of Sciences USA* 105: 1380–1385.

Palazón J, Cusidó R-M, Bonfill M, Mallol A, Moyano E, Morales C, Piñol M.T. 2003. Elicitation of different *Panax ginseng* transformed root phenotypes for an improved ginsenoside production. *Plant Physiology and Biochemistry.*41: 1019-1025.

Sivakumar G, Paek K. 2005. Methyl Jasmonate Induce Enhanced Production of Soluble Biophenols in PANAX GINSENG Adventitious Roots from Commercial Scale Bioreactors. *Chemistry of Natural Compounds* 41: 669-73.

Sengupta S, Toh S, Sellers L, Skepper J, Koolwijk P, et al. 2004. Modulating angiogenesis: the yin and the yang in ginseng. *Circulation* 110: 1219.

Son S-H, Choi S-M, Kwon S-R, Lee Y-H, Paek K-Y. 1999b. Large-scale culture of plant cell and tissue by bioreactor system. *Journal of Plant Biotechnology.* 1: 1-8.

Song I-J, Kang H-G, Kang J-Y, Kim H-D, Bae T-W, et al. 2009. Breeding of four-leaf white clover (Trifolium repens L.) through ^{60}Co gamma-ray irradiation. *Plant Biotechnology Reports* 3: 191-7.

Scott G, Colligan P, Ren B, Ren J. 2001. Ginsenosides Rb1 and Re decrease cardiac contraction in adult rat ventricular myocytes: role of nitric oxide. *British journal of pharmacology* 134: 1159.

Shinkai K, Akedo H, Mukai M, Imamura F, Isoai A, et al. 1996. Inhibition of in vitro Tumor

Cell Invasion by Ginsenoside Rg_3. *Cancer Science* 87: 357-62.

Subhan F, Anwar M, Ahmad N, Gulzar A, Siddiq A, et al. 2004. Effect of gamma radiation on growth and yield of barley under different nitrogen levels. *Pakistan Journal of Biological Sciences* 7: 981-3.

Souret FF, Kim Y, Wyslouzil BE, Wobbe KK, Weathers PJ. 2003. Scale-Up of *Artemisia annua* L. Hairy Root Cultures Produces Complex Patterns of Terpenoid Gene Expression. *Biotechnology and Bioengineering* 83: 653-67.

Tanaka O, Kasai R. Saponins of ginseng and related plants. 1984. In: Hertz W, Griesebach H, Kirby GW, Tamm C (editors), *Progress in the Chemistry of Organic Natural Products, New York: Springer-Verlag*. 46: 1-76.

Wang Z, Li M, Wu W, Tan H, Geng D. 2008. Ginsenoside Rb1 preconditioning protects against myocardial infarction after regional ischemia and reperfusion by activation of phosphatidylinositol-3-kinase signal transduction. *Cardiovascular Drugs and Therapy* 22: 443-52.

Wang W, Zhao Z, Xu Y, Qian X, Zhong J. 2006. Efficient induction of ginsenoside biosynthesis and alteration of ginsenoside heterogeneity in cell cultures of Panax notoginseng by using chemically synthesized 2-hydroxyethyl jasmonate. *Applied microbiology and biotechnology* 70: 298-307.

Wang Y,Wang BX, Liu TH, Minami M, Nagata T, Ikejima T. 2000. Metabolism of ginsenoside Rg1 by intestinal bacteria. II. Immunological activity of ginsenoside Rg1 and Rh1. *Acta Pharmacologica Sinica* 21: 792-796.

WU J-M, LIN H-Y, ZHAO L-H, JIA H-T, JIA H-K, et al. 2007. Comparative study on

quality of Tongrentang Red Ginseng and Korean Red Ginseng—determination of ginsenosides and polysaccharides. *China Journal of Chinese Materia Medica* 7.

Wu C-H, Murty H-N, Hahn E-J, Paek K-Y. Establishment of adventitious root co-culture of 2007. *Ginseng* and *Echinacea* for the production of secondary metabolites, Acta Physiologiae Plantarum, 30, 891-896.

Xue J-F, Liu Z-J, Hu J-F, Chen H, Zhang J-T, Chen N-H. 2006. Ginsenoside Rb1 promotes neurotransmitter release by modulating phosphorylation of synapsins through a cAMP-dependent protein kinase pathway. *Brain Research* 1106: 91-8

Xie J-T, Mehendale SR, Li X, Quigg R, Wang X, et al. 2005. Anti-diabetic effect of ginsenoside Re in ob/ob mice. Biochimica et Biophysica Acta (BBA) - *Molecular Basis of Disease* 1740: 319-25.

Xie J-T, Shao Z-H, Vanden Hoek TL, Chang W-T, Li J, et al. 2006. Antioxidant effects of ginsenoside Re in cardiomyocytes. *European Journal of Pharmacology* 532: 201-7.

Tian-min X, Ying X, Man-hua C, Xin J, Li-ping G. 2007. Inhibitory effect of ginsenoside Rg3 combined with cyclophosphamide on growth and angiogenesis of ovarian cancer. *Chinese medical journal* 120: 584-8.

Yokozawa T, Oura H. 1990. Facilitation of protein biosynthesis by ginseoside Rb2 administr4-1518 ation in diabetic rats. *Journal of Natural Products* 53: 1514-1518.

Yokozawa T, Yasui T, Oura H. 1993a. Effect of ginsenoside Rb2 on RNA synthesis. *Phytotherapy Research* 7: 68-77.

Yokozawa T, Yasui T, Oura H. 1993b. Stimulation of RNA polymerase activity by ginsenoside-Rb2 in diabetic rats. *Phytotherapy Research* 7: 240-243.

Yu K-W, Gao W, Hahn E-J, Paek K-Y. 2002. Jasmonic acid improves ginsenoside accumulation in adventitious root culture of *Panax ginseng* C.A. Meyer. *Biochemical Engineering Journal* 11: 211-5

Yu K-W, Gao W-Y, Son S-H, Paek K-Y. 2000. Improvement of ginsenoside production by jasmonic acid and some other elicitors in hairy root culture of ginseng (*Panax ginseng* C. A. Meyer). *In Vitro Cellular & Developmental Biology - Plant* 36: 424-8.

Yu L-C, Chen S-C, Chang W-C, Huang Y-C, Lin KM, et al. 2007. Stability of angiogenic agents, ginsenoside Rg1 and Re, isolated from *Panax ginseng*: In vitro and in vivo studies. *International Journal of Pharmaceutics* 328: 168-76.

Yokozawa T, Wu Liu Z. 2000. THE ROLE OF GINSENOSIDE-Rd IN CISPLATIN-INDUCED ACUTE RENAL FAILURE. *Renal Failure* 22: 115-27.

Yang Z-G, Sun H-X, Ye Y-P. 2006. Ginsenoside Rd from *Panax notoginseng* Is Cytotoxic towards HeLa Cancer Cells and Induces Apoptosis. *Chemistry & Biodiversity* 3: 187-97.

Zhou W, Chai H, Lin P, Lumsden A, Yao Q, Chen C. 2005. Ginsenoside Rb1 blocks homocysteine-induced endothelial dysfunction in porcine coronary arteries. J*ournal of vascular surgery* 41: 861-8.

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