Induction of Ginseng Hairy Roots And Their Possible Application To Large Scale Culture

Deok-Chun Yang*
College of Life Science, Kyung Hee University, Yongin 449-701, Korea

ABSTRACT

Ginseng(*Panax ginseng* C. A. Meyer) is important medicinal plant but requires 4-year cultivation for root harvest because of slow growth. In contrast, ginseng hairy roots induced by introducing Ri-plasmid of *Agrobacterium rhizogenes* into genomic DNA of plant cells show vigorous growth, and the hairy roots produce the same or more saponins than natural ginseng roots. Therefore, hairy roots can be used for commercial purposes. The present study was carried out to induce hairy roots with both active growth and high saponin contents. Numerous hairy roots of *Panax ginseng* were obtained after root disks of three-year old roots were infected with *Agrobacterium rhizogenes* R1000 A4T in dark condition after one month of culture. About 3 hundred lines of hairy roots were selected according as morphological characters on medium with carbenicillin. After pre-selection of fifteen lines of hairy roots with active growth, KGHR-1 and KGHR-8 lines were finally selected which had characters of high content of ginsenoside-Rd and ginsenoside-Re, respectively. The optimum growth of hairy roots was achieved in the culture of 1/2 MS liquid medium in dark (22℃) under 60 rpm gyratory shaking. Hairy roots grew well in 5L Erlenmeyer flasks, 1L roller drums, 10L jar-fermenters, and especially in 20L air-lift culture vessels.

Key words **:** Air-lift bioreactor, cell line, ginseng hairy roots, ginsenoside, large scale culture

INTRODUCTION

Korean ginseng is representative medicinal plants in Korea and have various pharmacological effects in human bodies. Lately, the ginseng is manufactured into various kinds of health foods, thus the demand is increased gradually. In addition, the ginseng saponins can be used for medicinal purpose. However, the cultivation of ginseng is very difficult and the field of ginseng cultivation is decreased gradually. Ginseng hairy roots induced by introducing T-DNA of A. *rhizogenes into genomic* DNA of plant cells show vigorous growth, and the hairy roots produce the same or more saponins than natural ginseng roots (Chilton et al., 1982; Tepfer, 1984; Yoshikawa and Furuya, 1987; Inomata et al. 1993). Therefore, mass production of ginseng saponin (ginsenosides) via *in vitro* culture can applied to new protocol.

Production of ginseng saponin (ginsenosides) from hairy roots induced by *Agrobacterium*- mediated transformation can be used for efficient method since the hairy roots are genetically stable and show active

Corresponding author : **Deok-Chun Yang,** (phone) 031-201-2688, (E-mail) dcyang@khu.ac.kr

growth potential in hormone-free medium(Choi et. al., 2001; Yang and Choi, 2000).

The present experiment is carried out to select the lines of hairy roots with active growth and to accomplish the mass production of bioactive metabolite (ginsenosides) for commercial application.

MATERIALS AND METHODS

Induction of hairy roots

Ginseng roots of 3-years old were surface-sterilized by NaOCl and pieced to root disks. The root disks were co-cultured with A. *rhizogenes* R1000 or A. *tumefaciens* A4T, then transferred to hormone-free MS(Murashige and Skoog, 1962) medium with 500㎍/mL carbenicillin at 25℃ in dark condition.

PCR analysis of *rol* C and *VirC* genes

To investigate the integration of T-DNA, hairy roots were sampled and the *rolC* (540bp) and *virC* genes were examined by PCR. DNA extraction and PCR analysis from the ginseng hairy roots was carried out according to the procedure of Edwards et al (1991) for DNA extraction, and that of Oono et al (1993), for PCR reaction. The primers for *rolC* DNA were 5`-ATGGCTGAAGACGACCTGTGTT-3` and 5`-TTAGCCGATTGCAAACTTGCAC-3`. The primers and the reaction buffers were incubated in a DNA thermal cycler(Perkin Elmer Cetus, Fotodyne Incorporated) using the following conditions: 96℃ for 2 min, followed by 36 cycles of 94℃ for 30 sec, 60℃ for 30 sec, 72℃ for 2 min, and final 15 min extension at 72 ℃.

To verify whether the hairy roots were truely transformant, *virC* genes were also observed by PCR analysis. The primers for *virC* DNA were 5` ATCATTTGTAGCGACT-3` and 5`-AGCTCAAAC CTGCTTC-3`; Sawada et al. 1995.

Content of ginsenosides

The content of ginsenosides was analysed by HPLC from n-BuOH extracts. Pump : Waters(U6K), column : Lichrosorb-NH2 column(10㎛, Merk), detector : Waters R401 differential refractometer, solvent : acetonitrile: H_2O: n-BuOH (80:20:10, v/v/v).

Mass culture of hairy roots

Hairy roots were cultured in 100mL, 250mL Erlenmeyer flask, and 2.5L, 5L and 30L air lift incubators, and 10L jar fermenter for 2 month culture cycle.

RESULTS AND DISCUSSION

Induction of hairy roots

After co-culture of root disks with *Agrobacterium rhizogenes* R1000 or A. *tumefaciens* A_4T, hairy roots were induced as shown in Figure 1. About 3 hundred lines of hairy roots were selected and maintained by root tip culture.

All the hairy roots were identified to transformed roots by PCR analysis. To confirm whether the PCR products of hairy roots were the results from true transformant or from contamination of A. *rhizogenes*, PCR analysis was performed using both *rolC* primers and *virC* primers(Figure 2). Agarose gel electrophoresis revealed a band of approximately 540 bp that corresponded to a fragment of *rolC* gene only in the mixture prepared with DNA from A. *rhizogenes*. Whereas, the *virC* gene, not transmitted in the plant genome, was not detected all of hairy roots(Figure 2). This indicates that the hairy roots were transformed and showed no contamination of A. *rhizogenes*.

Selection of actively growing hairy roots

Among selected 3 hundred hairy roots, 15 lines with active growing characters were selected. Thereafter, 7 lines were finally selected, the growth rate of them

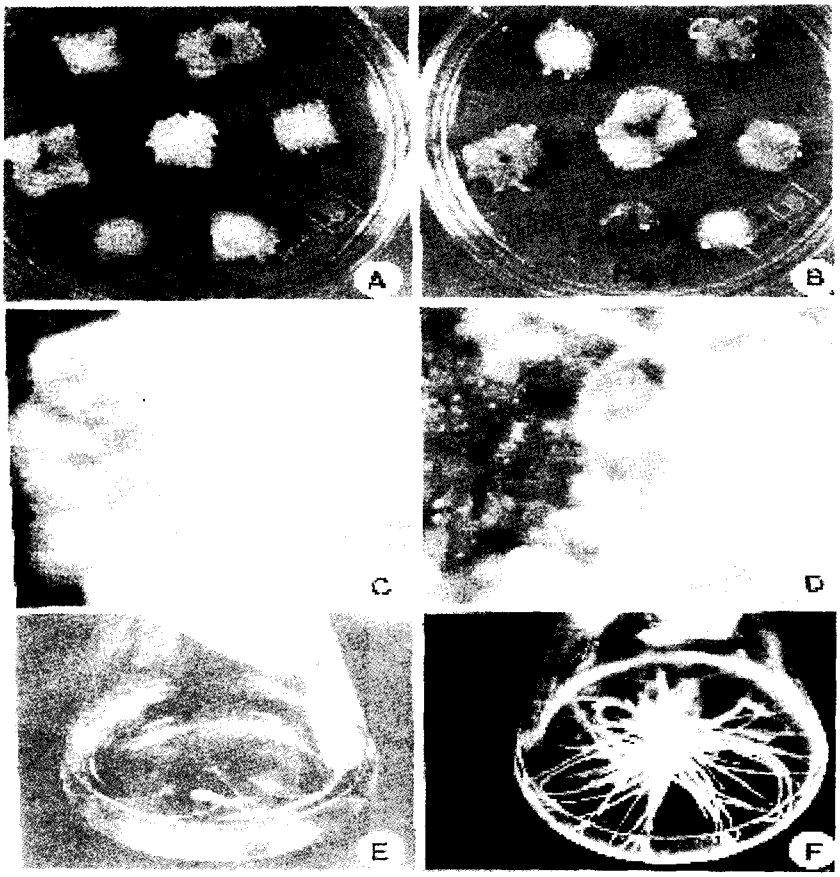

Fig. 1. Induction of ginseng hairy roots from Agrobacterium-mediated transformation. A-B; Hairy roots were protruded from the surfaces of root disks after 30 days. C-D; Hairy roots after 40 days. E-F; Hairy roots in liquid culture after culture of root tips of hairy roots after 30 days.

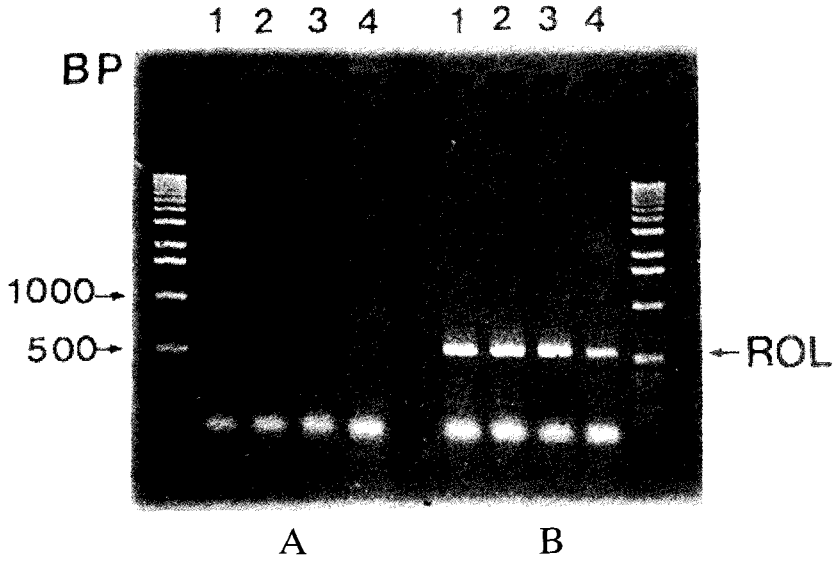

Fig. 2. PCR products of virC(A) and rolC gene(B, 540bp) from ginseng hairy roots.

showed 1.5 to 2 times compared to control, especially in KGHR-1, KGHR-5, KGHR-8 (Figure. 3).

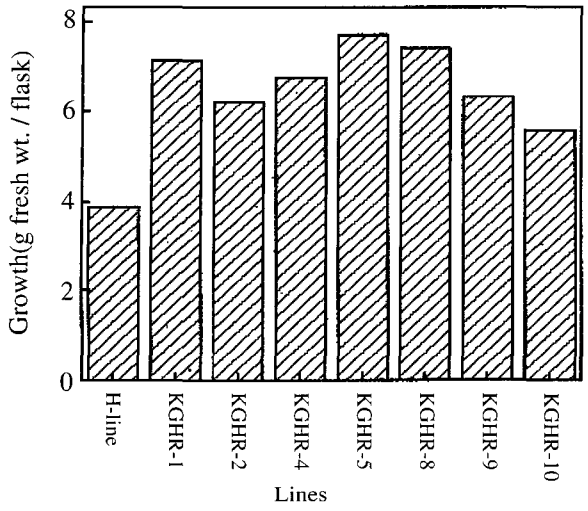

Fig. 3. Selection of hairy root lines(KGHR-1, KGHR-5, KGHR-8) with active growth.

Selection of hairy roots with high content of ginsenosides

After culture of 12 lines of hairy roots on 1/2MS agar medium for 50 days, three lines of hairy roots with high content of ginsenoside were selected, KGHR-1, KGHR-5, KGHR-8 (Figure 4), they have 4 times of ginsenoside compared to the control (Table 1). Conclusively, the selected three lines of ginseng hairy roots (KGHR-1, KGHR-5, and KGHR-8) can be applied to mass production of ginsenosides in pilot system for commercial scale.

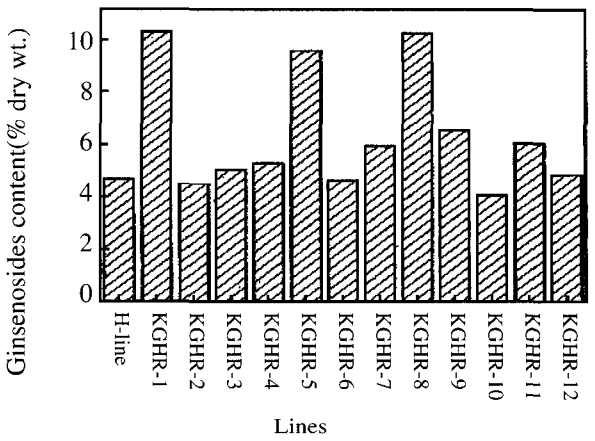

Fig. 4. Ginsenoside contents of superior lines of ginseng hairy roots derived from somaclonal variation.

Effect of inoculation contents on the growth of hairy roots

The optimum condition for hairy root culture in liquid medium was 1/2MS medium for 30 days at 22℃ in the dark. When the hairy roots were inoculated at various levels (1g to 6g) for 30 days of culture, the highest yield of growth was achieved when 1g of hairy roots were inoculated (Table 2).

Effect of culture type on the growth of hairy roots

Hairy roots (KGHR-8) were grown well in the various culture system (Figure 5). When the 50g of hairy roots were cultured in the 30L air lift culture vessel with 20L culture medium, about 1,500g/FW of hairy roots were gained after 2 months of culture (30 times of growth yield).

Table 1. Productivity of ginsenoside from superior ginseng hairy root.

Cell lines	Ginsenosides(A) (mg/ g dry wt.)	Growth(B) (g fresh wt./flask)	Productivity(AB) of ginsenoside(mg / flask)
H-line	4.70	3.89	18.28
KGHR-1	10.32	7.13	73.58
KGHR-5	8.56	7.72	73.80
KGHR-8	10.25	7.42	76.06

Table 2. Growth and productivity of ginseng hairy root according to initial inoculum size

Inoculum size(I) (g)	Growth Fresh wt.(F) (g / 100 mL)	Productivity Dry wt.(D) (g / 400 mL)	F/I	D/I
1 (1.09)	5.61 °æ 0.168	2.03	5.61	1.86
2 (2.05)	8.38 °æ 0.548	2.91	4.19	1.42
3 (2.84)	9.99 °æ 0.474	3.19	3.33	1.23
4 (3.96)	12.26 °æ 1.627	3.68	3.06	0.93
5 (5.23)	14.39 °æ 0.554	4.25	2.88	0.81
6 (6.12)	14.71 °æ 0.867	4.34	2.45	0.69

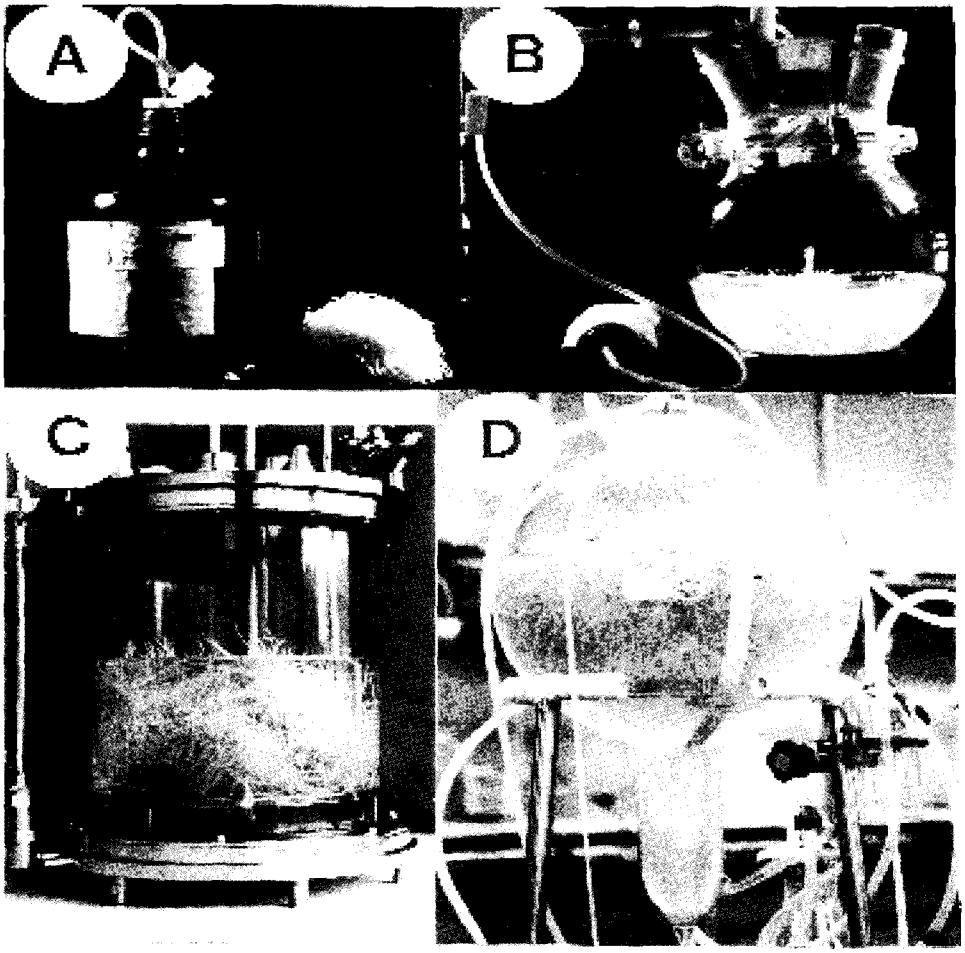

Fig. 5. Hairy roots cultured in various types of culture vessels. A; 2.5L aeration culture vessel. B; 5L vessels with aeration and ventilation system. C; 10L jar fermenter. D; 30L balloon-typed vessel.

Table 3. Growth of ginseng hairy roots on the various culture vessels

Vessel	Media volumn	Initial inoculum (g fresh wt.)	Growth (g fresh wt.)	Time(Months)	Air supply
100 mL flask	40 ml	1	8	1	shaking
250 mL flask	100 ml	4	30	1	shaking
2.5 L air lift	1 L	10	200	2	air lift
5 L air lift	2 L	20	450	2	air lift
10 L jar fermenter	5 L	20	600	3	air lift
30L air lift	20 L	50	1,500	2	air lift

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