Chapter 11
Production of Ginsenosides by Hairy Root Cultures of *Panax ginseng*

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Abstract *Panax ginseng* C. A. Meyer, commonly known as ginseng, is a popular medicinal plant, used as a traditional medicine in many countries. Ginsenosides are triterpene compounds which have multiple biological and pharmaceutical applications including neuroprotection, anticancer, antidiabetic, hepatoprotective, and immunomodulatory activities. Cultivation of ginseng till harvest of matured roots takes 5–7 years, whereas wild ginseng is rare and a highly expensive commodity. Therefore, many researchers studied biotechnological means especially cell and organ cultures for the production of ginsenosides. Transformed hairy roots were induced in ginseng, and they were cultured in vitro for biomass and secondary metabolite production. Various chemical and physical parameters have been worked out for optimal biomass and ginsenoside accumulation. Several researchers have tested bioreactor system for cultivation of ginseng hairy roots to produce ginsenosides. This review highlights the recent progress in the hairy root induction, selection of elite clones, establishment of suspension culture, strategies adopted for biomass, and bioactive compound production.

Keywords Biomass • Elicitor • Saponins • Secondary metabolite • Suspension cultures

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Introduction

Panax ginseng C. A. Meyer, commonly known as "ginseng," is a medicinal plant which is cultivated in Korea, China, Japan, and other Southeast Asian countries and has been used as an herbal medicine in these countries for thousands of years. These days it is also popular as a nutraceutical or as a functional food in the rest of the world especially in North America and Europe (Murthy et al. 2014a). It exhibits pharmacological effect including antifatigue, anticancer, antidiabetic, cardioprotective, hepatoprotective, immunomodulatory, and antioxidant properties (Briskin 2000; Park et al. 2005; Murthy et al. 2014b, c). The biologically active components of ginseng are triterpenoid saponins, known as ginsenosides. Ginsenosides are classified into three groups based on their structure, namely, the Rb group (panaxadiols, including Rb_1, Rb_2, Rc, Rd, and so forth), Rg group (panaxatriols, including Rg_1, Re, Rf, Rg_2, and so forth), and Ro group (oleanolic acid) (Fig. 1; Park et al. 2005). Ginseng roots which are naturally available are very scarce; the current ginseng supply depends almost exclusively on field cultivation which is time-consuming and labor-intensive. Agricultural production of ginseng roots requires 5–7 years from seed planting to mature root harvesting during which the plant growth is highly susceptible to a number of environmental factors, pathogens, and pests (Proctor 1996). Therefore, biotechnological methods such as cell, tissue, and organ cultures have been exploited for ginseng biomass and its bioactive constituent production (Wu and Zhong 1999; Murthy et al. 2016, 2014a, d; Thanh et al. 2014a, b). Many investigators have studied the production of ginsenosides using callus tissue, cell suspension cultures, but the productivity obtained has remained low because of low growth rates (Furuya et al. 1983; Mathur et al. 1994). Additionally, the induction and establishment of hairy roots after the infection of *Panax ginseng* roots in *Agrobacterium rhizogenes* have been successfully performed (Inomata et al. 1993; Mallol et al. 2001; Sivakumar et al. 2005; Yoshikawa and Furuya 1987). There are various reports on ginseng hairy root cultures focusing on the increase of biomass growth and ginsenoside productivity by the optimization of culture medium and environment (Kim et al. 2013; Palazon et al. 2003a, b; Sivakumar et al. 2005; Yu et al. 2000, 2005). It is also possible to enhance the productivity of ginsenosides by application of bioreactor technologies (Choi et al. 2005; Sivakumar et al. 2005; Yu et al. 2000, 2005; Palazon et al. 2003b; Jeong and Park 2005, 2006; Jeong et al. 2003). This review summarizes the methods of ginseng hairy root cultures for the production of biomass and ginsenosides.

Induction of Hairy Roots in *Panax ginseng*

Hairy roots from ginseng were induced from root-derived callus following infection with *Agrobacterium rhizogenes* strain A4 (Yoshikawa and Furuya 1987), from root discs using *A. rhizogenes* strain A4 (Hwang et al. 1999; Mallol et al. 2001), R-1000 (Woo et al. 2004), KCTC-2703 (Yu et al. 2000), and from petiole segments with *A. rhizogenes* strain 15834 (Yoshimatsu et al. 1996). Root discs developed both callus tissues and hairy roots after 4–8 weeks of infection with *A. rhizogenes* strain A4. Various hairy root lines were recognized with respect to morphology and growth

(Mallol et al. 2001): HR-M line was with primary roots, extensive lateral branching, and a profusion of root hairs, C-M line showed callus-like appearance with very thick primary roots and several secondary roots, and T-M line was long and thin without branching points. Hairy roots lines were maintained in Schenk and Hilderbrandt (1972) liquid medium and kept in a rotary shaker at 100 rpm, 26 °C in the dark by subculturing them once after 2 weeks. They could able to maintain these three root phenotypes over successive subcultures for 2 years. Hairy root lines showed differential growth rate (final fresh weight/fresh inoculum weight)—highest growth values were 7.9, 7.1 and 3.2, respectively, by C-M, HR-M, and T-M roots, and growth rate was 1.5 with respect to non-transformed roots. Hairy roots were found to be superior in growth and accumulation of biomass in suspension cultures, and such reports are already on records in hairy root cultures of cucumber (Amselem and Tepfer 1992), rosy periwinkle (Palazon et al. 1998), jimsonweed, and tobacco (Moyano et al. 1999).

Selection of Clones/Lines for Ginsenoside Accumulation

Of the three hairy root lines selected by Mallol et al. (2001), HR-M root lines achieved the highest ginsenoside content (5.4 mg/g DW), whereas C-M line accumulated 4.8 mg/g DW and non-transformed roots possessed 4.5 mg/g DW. Both Rb and Rg group ginsenosides were produced by these hairy root lines; Rg group ginsenosides were always higher than that of the Rb group. The ginsenoside pattern also varied depending on the root phenotypes. The main ginsenosides found in T-M, HR-M, and C-M root lines were the Re ginsenosides followed by the Rg_1. In T-M root the Re and Rg_1 represented 57.7 and 16.8%, respectively, of the total ginsenoside contents; in HR-M and C-M root lines, the Re constituted 40.1 and 38.1%, and the ginsenoside Rg_1 represented 28.5 and 27.9%, respectively, of the total. With respect to ginsenoside yield (after 4 weeks of suspension culture), Mallol et al. (2001) observed highest yield of 7.29 mg/l by HR-M root lines, followed by C-M root lines (71.6 mg/l). The lowest ginsenoside yield of transformed roots phenotypes was with T-M root lines (24.8 mg/l). In concurrence with these observations, Woo et al. (2004) and Yu et al. (2000) also selected hairy root lines producing total ginsenoside contents 4–5 times higher than that of a common hairy root population.

Ginseng Hairy Root Culture: Optimization of Factors Influencing Biomass and Metabolite Production

Effect of Growth Regulators on Biomass and Metabolite Production

Yu et al. (2000) used hormone-free Murashige and Skoog (1962) liquid medium for establishment of hairy root suspension cultures; whereas, Mallol et al. (2001) have used SH liquid medium for multiplication and growth of ginseng hairy roots.

The ginseng hairy root cultures have been thus established with the hairy root lines selected by various laboratories. These hairy roots were stable in culture, and the growth rate was much faster than that of non-transformed roots in hormone-free medium. The hairy roots grew rapidly in a hormone-free medium and showed biphasic growth: rapid growth during first 2 weeks and slower growth thereafter (Yu et al. 2000). Thus ginseng hairy root suspension cultures need 4–6 weeks depending on the hairy root clones/lines to achieve growth, biomass, and metabolite accumulation. The 0.5 g fresh weight of ginseng hairy roots increased ca. 100-fold after 6 weeks of culture in hormone-free medium. Hwang et al. (1999) studied the effect of exogenous auxins including indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), naphthalene acetic acid (NAA), and 2, 4-dichlorophenoxy acetic acid (2, 4-D) on growth and ginsenoside accumulation, and they reported that IAA at 0.5 mg/l was most effective hormone for promotion of growth, multiplication, and accumulation of ginsenosides. The increase of root growth in ginseng was the result of an increase in branching and root elongation. Similarly, in case of *Rubia tinctorum*, hairy roots cultured in presence of IAA showed the maximal growth rate and the highest alkaloid production. In contrast, Inomata et al. (1993) showed that the addition of IBA with ginseng hairy root cultures increased the growth ratio and ginsenoside accumulation. Another study demonstrated that the exogenous IAA had no great effect on hairy root growth or the production of alkaloids (Rhodes et al. 1994). Hwang et al. (1999) showed accumulation of higher levels of ginsenosides when 0.5 mg/l gibberellic acid (GA) was added to the medium. However, in the hairy root cultures of *Artemisia annua*, GA greatly stimulated the increase of fresh weight, mainly due to the enhanced branching but inhibited the production of alkaloids (Liu et al. 1997). Hwang et al. (1999) also studied the effect of putrescine, spermidine, and spermine on growth and ginsenoside content, and these hormones did not promote growth of hairy roots or accumulation of ginsenosides. In case of the roots of *Duboisia myoporoides*, the addition of putrescine and spermidine led to the increased production of scopolamine (Yoshika et al. 1989). Therefore, the differential effect of phytohormones on growth and secondary metabolite accumulation is thought to be related to the genotype and physicochemical characteristics of the explants.

Effect of Medium Salt Strength on Biomass and Metabolite Production

The culture medium and salt strength influence the growth, physiology, and metabolism of in vitro cultured explants (Murthy et al. 2014e). Therefore, the selection appropriate medium and salt concentration of culture medium are very much essential. Sivakumar et al. (2005) studied the effect of 0.50, 0.75, 1.0, and 1.50 salt strength of MS medium on hairy root growth and ginsenoside productivity. Salt strength of 0.75 was optimal for ginseng hairy root growth as compared to other

Table 11.1 Optimization of salt strength of Murashige and Skoog medium for ginseng hairy root growth and ginsenoside production

Salt strength	Biomass		Percentage DW	Growth rate	Ginsenoside (mg/g)	Ginsenoside yield (mg/l)
	Fresh weight (g)	Dry weight (g)				
0.50	15.6 ± 0.43	1.22 ± 0.02	7.82	5.73	13.20 ± 0.25	161.04
0.75	18.7 ± 0.30	1.37 ± 0.01	7.33	6.43	9.78 ± 0.30	133.99
1.00	16.9 ± 0.12	1.20 ± 0.01	7.10	5.63	6.82 ± 0.81	81.84
1.50	14.2 ± 0.50	1.04 ± 0.2	7.32	4.88	5.65 ± 1.12	58.76

Data collected after 5 weeks culture using 400 ml of conical flask containing 100 ml of MS medium

Growth rate was calculated from increase in dry weight. Values are quotients of dry weight after cultivation and dry weight of the inoculum

Mean values are with standard error of three replicates

media (growth rate 6.43; Table 11.1), whereas, 0.5 strength MS medium was responsible for maximum ginsenoside production (161.04 mg/l; Table 11.1). A full strength MS medium was suitable for cell suspension cultures of *Gymnema sylvestre* for biomass accumulation and gymnemic acid production (Nagella et al. 2011). Similarly, a full strength MS medium was appropriate for growth of cells and secondary metabolite accumulation in *Withania somnifera* (Praveen and Murthy 2010).

Effect of Carbohydrates on Biomass and Metabolite Production

Sucrose, glucose, fructose, glucose + fructose, and sucrose + glucose were tested on ginseng hairy root growth and ginsenoside productivity by Sivakumar et al. (2005), and they found sucrose as a suitable carbohydrate. They also verified the effect of different concentrations of sucrose in range 1–9% and reported that hairy root growth, biomass accumulation increased with increase in sucrose concentration, whereas low sucrose concentration such as 2% was suitable for ginsenoside production (Table 11.2). The maximum ginsenoside content (8.01 mg/g DW) and ginsenoside productivity were obtained with the 2% sucrose. Sucrose at 1–3% was favorable for both Rg and Rb group of ginsenosides. Among various ginsenosides, the contents of Rg_1, Rb_1, Rb_2, and Rd decreased compared to other ginsenosides (Table 11.3). Based on such results, they followed two-stage culture system for ginseng hairy root culture; during the growth stage, higher sucrose concentration was used, while during the ginsenoside production stage, a relatively lower concentration of sucrose (2%) was maintained. The level of sucrose has been shown to affect the growth, development, and metabolism of transformed roots (Wang and Weathers 2007). For instance, 3% sucrose was found to be optimal for biomass accumulation, and 4% sucrose favored the production withanolide A in the tested concentrations (1–8%) with hairy root cultures of *Withania somnifera* (Praveen and Murthy 2012). Similarly, 4–6% of

Table 11.2 Effect of sucrose concentration in Murashige and Skoog medium on ginseng hairy root growth and ginsenoside production

Sucrose (%)	Biomass		Percentage dry weight	Growth rate	Ginsenosides (mg/g dry weight)			Ginsenoside yield (mg/l)
	Fresh weight (g)	Dry weight (g)			Rg	Rb	Total	
1	15.40 ± 0.04	0.69 ± 0.01	4.51	4.14	3.00	4.13	4.13	49.20
2	21.50 ± 0.50	1.30 ± 0.22	5.59	7.74	2.80	5.22	5.22	104.13
3	23.29 ± 0.40	1.31 ± 0.01	5.63	7.80	3.20	5.30	5.30	85.54
5	22.50 ± 0.03	1.83 ± 0.01	8.15	10.92	1.70	3.28	3.28	91.13
7	21.10 ± 0.80	1.90 ± 0.05	8.60	11.31	1.12	1.56	1.56	50.73
9	19.80 ± 0.05	2.09 ± 0.06	10.56	12.44	1.02	0.74	0.74	36.99

Data collected after 5 weeks culture using 400 ml of conical flask containing 100 ml of MS medium
Growth rate was calculated from increase in dry weight. Values are quotients of dry weight after cultivation and dry weight of the inoculum
Mean values are with standard error of three replicates

Table 11.3 Effect of sucrose concentration supplemented to Murashige and Skoog medium on accumulation of ginsenosides in ginseng hairy roots

Sucrose (%)	Ginsenosides (mg/g dry weight)							Ratio of Rb/Rg
	Rg group			Rb group				
	Rg_1	Re	Rf	Rb_1	Rc	Rb_2	Rd	
1	2.54	0.32	0.12	2.27	0.62	0.77	0.47	1.38
2	2.50	0.21	0.08	2.24	0.77	1.09	1.06	1.86
3	2.29	0.19	0.05	1.30	0.54	1.18	1.01	1.66
5	1.53	0.13	0.04	1.09	0.61	0.91	0.67	1.93
7	0.99	0.09	0.04	0.59	0.30	0.44	0.23	1.39
9	–	0.08	0.05	0.14	0.31	0.03	0.27	0.73

Data collected after 5 weeks culture using 400 ml of conical flask containing 100 ml of MS medium
Mean values are with standard error of three replicates

sucrose was found to be optimal for accumulation of steroidal alkaloids in hairy root cultures of *Solanum aviculare* (Yu et al. 1996), and 3% of sucrose was beneficial for accumulation of gymnemic acid with hairy root cultures of *Gymnema sylvestre* (Nagella et al. 2011), respectively.

Effect of pH on Biomass and Metabolite Production

The hydrogen ion concentration of the culture medium is also one of the factors influencing the growth of cultured cells and organs and productivity of secondary metabolites (Murthy et al. 2014e). The medium pH is usually set at 5.6, and extreme pH values are avoided. The concentration of hydrogen ions in the medium changes

Table 11.4 Influence of medium pH on hairy root growth and ginsenoside production

pH	Biomass dry weight (g)	Growth rate	Ginsenosides (mg/g dry weight)	Ginsenoside yield (mg/l)
3.0	1.07 ± 0.03	6.37	15.6 ± 0.98	166.92
4.0	1.14 ± 0.08	6.77	17.0 ± 2.26	193.80
5.0	1.18 ± 0.02	7.01	18.5 ± 0.35	218.30
6.0	1.25 ± 0.01	7.44	19.1 ± 0.56	238.85
6.5	1.21 ± 0.02	7.22	19.8 ± 0.98	239.68
7.0	1.02 ± 0.02	6.01	13.2 ± 1.10	134.64

Data collected after 5 weeks culture using 400 ml of conical flask containing 100 ml of MS medium
Growth rate was calculated from increase in dry weight. Values are quotients of dry weight after cultivation and dry weight of the inoculum
Mean values are with standard error of three replicates

during culture period; this is due to uptake of nutrients by the cultured explants or the accumulation of metabolites (McDonald and Jackman 1989). Sivakumar et al. (2005) studied the effects of initial medium pH on ginseng hairy root growth and ginsenoside production in MS medium, and they reported that the maximum growth rate (7.44) and optimal ginsenoside productivity (239.68 mg/l) were obtained at 6.0 and 6.5, respectively. They have observed the inhibition of hairy root growth and ginsenoside production when initial pH was below 4.0 or above 7.0 (Table 11.4). In *Withania somnifera* hairy root cultures, the initial medium pH 5.8 favored biomass accumulation (12.1 g/l DW), and a medium pH of 6.0 favored accumulation of withanolide A in roots (13.84 mg/g DW; Praveen and Murthy 2012). In hairy root cultures of *Tagetes patula*, a medium pH of 5.7 was suitable for the growth and accumulation of thiophene (Mukundan and Hjortso 1991).

Effect of Temperature and Light on Biomass and Metabolite Production

Temperature and light are the bioprocess parameters affecting suspension cultures (Murthy et al. 2014e). It has been shown that the optimal temperature treatment of suspension cultures is necessary for accumulation of biomass and production of metabolites (ten Hoopen et al. 2002; Zhong and Yoshida 1993). The stimulatory effect of light on biomass growth and formation of secondary metabolites was shown in red beet (*Beta vulgaris*; Shin et al. 2004) and Chinese basil (*Ocimum basilicum*; Zhong et al. 1991). Whereas, light has an inhibitory effect on metabolite accumulation in purple gromwell (*Lithospermum erythrorhizon*; Tabata et al. 1974). Yu et al. (2005) verified the effect of temperature on ginseng hairy root growth under different temperature regimes 13/20, 20/13, 25/25, and 30/25 °C day and night cycles and obtained highest hairy root growth with cultures incubated at

Table 11.5 Effect of incubation temperature (with 16 h/8 h day/night cycles) on growth and ginsenoside production of ginseng hairy roots cultivated in 5 l bioreactors containing 4 l of medium for 4 weeks

Growth temperature (°C)	Biomass		Growth rate	Ginsenoside (mg/g dry weight)	Ginsenoside yield (mg/l)
	Fresh weight (g)	Dry weight (g)			
13/20	431 ± 1.0	28 ± 1.0	19.7	4.5 ± 0.1	31.5 ± 1.5
20/13	892 ± 0.9	65 ± 0.8	45.8	8.2 ± 0.1	133.9 ± 0.9
25/25	889 ± 0.6	51 ± 0.7	35.9	10.5 ± 0.1	133.4 ± 1.2
30/25	764 ± 0.8	64 ± 0.9	45.1	6.4 ± 0.1	71.6 ± 0.5

Mean values are with standard error of three replicates
Growth rate was calculated from increase in dry weight. Values are quotients of dry weight after cultivation and dry weight of the inoculum

Table 11.6 Effect of light quality on growth and ginsenoside production of ginseng hairy roots cultivated in 5 l bioreactors containing 4 l of medium for 4 weeks

Light source	Biomass		Growth rate	Ginsenosides (mg/g dry weight)			Ginsenoside yield (mg/l)
	Fresh weight (g)	Dry weight (g)		Rg	Rb	Rb/Rg	
Dark	270 ± 1.0	24 ± 0.6	11.4	2.8 ± 0.2	4.5 ± 0.2	1.6 ± 0.1	27.8 ± 1.0
Fluorescent light	226 ± 0.8	21 ± 0.6	10.1	5.3 ± 0.1	3.7 ± 0.7	0.7 ± 0.1	30.2 ± 0.9
Metal halide light	193 ± 1.1	19 ± 0.3	8.9	3.5 ± 0.4	3.4 ± 0.3	0.9 ± 0.2	23.3 ± 0.2
Blue light	236 ± 0.2	24 ± 0.9	11.3	3.8 ± 0.4	3.9 ± 0.5	1.0 ± 0.1	26.6 ± 0.4
Red light	284 ± 0.9	25 ± 1.0	11.6	3.1 ± 0.8	4.1 ± 0.7	1.3 ± 0.1	20.9 ± 0.4
Blue plus red lights	183 ± 0.9	21 ± 0.9	10.1	3.4 ± 0.1	2.9 ± 0.2	0.8 ± 0.2	24.2 ± 0.7

Mean values are with standard error of three replicates
Growth rate was calculated from increase in dry weight. Values are quotients of dry weight after cultivation and dry weight of the inoculum

20/13 °C, whereas highest ginsenoside accumulation was with cultures incubated at 25/25 °C (10.5 mg/g DW; Table 11.5). Likewise, Yu et al. (2005) tested the effect of fluorescent light, metal halide light, blue light, red light, and blue plus red light on growth and accumulation of ginsenosides in ginseng hairy root cultures and showed optimal growth of ginseng hairy roots with red light treatment (Table 11.6). They observed positive effect of fluorescent light on accumulation of ginsenosides (9.0 mg/g DW) compared to dark treatment (8.4 mg/g DW; Table 11.6). They also detected differential accumulation of Rb and Rg groups of ginsenosides with dark or light treatments. Rb group ginsenosides were highest in the dark-grown cultures (4.5 mg/g DW), and the production of Rg group ginsenosides was optimal in the light-grown cultures (fluorescent light, 5.3 mg/g DW). Therefore, control of ginsenoside accumulation in hairy root cultures is possible with varied light and dark treatments.

Effect of Elicitors on Biomass and Metabolite Production

Hairy root cultures have been established in various laboratories for the production of ginsenosides; however, the ginsenoside content in these hairy root lines was consistently low. Therefore, elicitation treatment of hairy root cultures has been widely studied for the overproduction of ginsenosides. Yu et al. (2000) tested effect of jasmonic acid as an elicitor in the range of 1.0–5.0 mg/l. Jasmonic acid strongly inhibited ginseng hairy root growth and biomass accumulation; however, it strongly improved ginsenoside production (Table 11.7). They have reported fourfold increment in ginsenoside content (58.65 mg/g DW), when compared to control (15.85 mg/g DW). Among ginsenosides, the Rb group showed an increase, while the Rg group was stable (Table 11.8). Rb_1 and Rb_2 ginsenosides increased 4.6 and 7.7 times, respectively, whereas other ginsenosides increased marginally as compared to control (Table 11.8). Based on their results, Yu et al. (2000) suggested two-stage culture system for ginseng hairy root culture. In the first stage a medium

Table 11.7 Effect of jasmonic acid on growth and ginsenoside production of ginseng hairy roots after 5 weeks of culture

Jasmonic acid (mg/l)	Biomass			Ginsenosides (mg/g dry weight)			Ratio of Rb/Rg	Ginsenoside yield (mg/l)
	Fresh weight (g)	Dry weight (g)	Growth rate	Rb	Rg	Total		
0.0	30.2a	1.52a	7.12	10.31d	5.51a	15.85d	1.92d	240.92d
1.0	24.5b	1.31b	6.12	30.08c	5.87a	35.98c	5.14c	471.34c
2.0	20.0c	1.08c	5.04	41.59b	6.05a	47.69b	7.24b	515.05b
5.0	14.1d	0.86d	4.04	59.98a	5.60a	58.65a	9.28a	504.39a

Mean values followed by different letters within a column are significantly different at $P \leq 0.05$ by Duncan's multiple range test. Each treatment was repeated three times

Growth rate was calculated from increase in dry weight. Values are quotients of dry weight after cultivation and dry weight of the inoculum

Table 11.8 Effect of jasmonic acid on production of different ginsenosides in ginseng hairy roots after 5 weeks of culture

Jasmonic acid (mg/l)	Ginsenosides (mg/g dry weight)							
	Rg group			Rb group				Total
	Rg_1	Re	Rf	Rb_1	Rc	Rb_2	Rd	
0.0	1.63ab	3.02a	0.87a	7.23d	1.20b	1.13d	0.11c	15.85d
1.0	1.85a	3.49a	0.52a	18.99c	5.56a	3.34c	2.3b	35.98c
2.0	1.73ab	3.69a	0.63a	24.09b	7.26a	4.62b	4.6a	47.69b
5.0	1.34b	3.74a	0.61a	33.70a	6.19a	8.80a	4.3a	58.65a

Mean values followed by different letters within a column are significantly different at $P \leq 0.05$ by Duncan's multiple range test. Each treatment was repeated three times

without elicitor facilitates the growth of hairy roots, while in the second stage the hairy root biomass would be transferred to fresh medium containing jasmonic acid, which triggers the accumulation of ginsenoside content. Palazon et al. (2003a) followed such suggestions and established ginseng hairy root cultures using selected clones, namely, C-M, HR-M, and T-M. They introduced methyl jasmonate (22.4 mg/l) as elicitor during progressive declaration growth phase, i.e., on day 25 of culture and obtained positive response. The root lines C-M, HR-M, and T-M accumulated 2, 1.8, and 4 times higher ginsenoside content compared to control cultures with elicitor treatment. Kim et al. (2013) reported the accumulation of novel ginsenoside such as Rg_3, with methyl jasmonate treatment of ginseng hairy root culture, and this metabolite is not present naturally in ginseng. Choi et al. (2005) and Kim et al. (2009) utilized the ginseng hairy root cultures which were treated with methyl jasmonate for the analysis of gene transcripts and to identify genes involved in biosynthesis of ginsenosides. Liang et al. (2015) evaluated the effect of Tween 80 permeabilization on ginsenoside secretion in *Panax ginseng* hairy root cultures and reported that with the use of 1.2% (w/v) Tween 80 for 25 days; approximately 76% of the total ginsenosides was released into the surrounding medium. Recently, Zhang et al. (2015) cloned α-L-rhamnosidase gene from *Bifidobacterium breve* into ginseng hairy roots for the enhanced accumulation of Rg_1 ginsenoside in the hairy roots. Ge et al. (2014) tested the efficacy of ginseng hairy roots in biotransformation and produced novel alkaloidal glycosides using tetrahydroprotoberberines as substrates. Similarly, Chen et al. (2008) used hairy roots of ginseng for regioselective glycosylation of hydroxybenzoic acids into their glycosides and glycosyl esters.

Establishment of Hairy Root Suspension Cultures in Bioreactors

Jeong et al. (2003) tested the growth characteristics of ginseng hairy roots in various bioreactors such as stirred bioreactors (1-l capacity with 800 ml working volume) and bubble column bioreactors (3-l, 5-l and 19-l capacity with 2.5-l, 4-l and 17-l working volume, respectively) and obtained hairy root growth of about 55-fold of inoculum after 39 days in 5-l bioreactor and 38-fold of inoculum after 40 days in a 19-l bioreactor. Palazon et al. (2003b) tested the effect of three variables, namely, the bioreactor system (2-l wave or 3-l spray reactor), medium exchange, and culture period of ginseng hairy roots (line T12), for the production of ginsenosides. Among the rectors, the wave bioreactor found to be more efficient in promoting hairy root growth. In wave reactor with medium exchange every 14 days over a culture period of 56 days, there was 28-fold increment of inoculum, giving a root biomass of 284.9 g/l and a ginsenoside content 145.6 mg/l. Yu et al. (2003) tested 10 l drum-type airlift bioreactors containing 8 l of working capacity with

aeration rate of 0.1 vvm (air volume/medium volume/minute) for cultivation of ginseng hairy roots and obtained fresh biomass 1670 g of fresh biomass (40 g of initial inoculum) and 109 g of dry biomass with growth yield of 76.8. The total ginsenoside content was 14.65 mg/g DW with ginsenoside productivity of 199.6 mg/l. Thus cultivation of ginseng hairy roots in airlift bioreactors is highly promising for the production of ginsenosides.

Conclusion and Future Perspectives

Hairy root cultures of ginseng have demonstrated great promise in terms of biomass accumulation and production of ginsenosides. Research developments on ginseng hairy root cultures have demonstrated that selection of superior clones/lines, establishment of suspension culture, optimization medium ingredients, culture conditions, and elicitation have been worked out successfully. Even bioreactor cultures have been initiated; however, important parameters such as selection of suitable bioreactor type, inoculum density, agitation/aeration, nutrient feeding, and precursor feeding have not been worked out, and future research efforts should be focused in these areas. Assessment of ginsenoside biosynthetic pathway and application of metabolic engineering are also desirable to obtain useful metabolites from ginseng hairy root cultures.

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