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MINI-REVIEW

Ginsenosides: prospective for sustainable biotechnological production

Hosakatte Niranjana Murthy · Milen I. Georgiev · Yun-Soo Kim · Cheol-Seung Jeong · Sun-Ja Kim · So-Young Park · Kee-Yoeup Paek

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Abstract *Panax ginseng* C.A. Meyer (ginseng) is a well-known medicinal plant that has been traditionally used in the oriental countries for centuries. Wild ginseng is a scarce and rare commodity. Field cultivation of the ginseng plant is a time-consuming and labor-intensive process. Ginsenosides, a group of glycosylated triterpenes, also known as saponins, are the principal bioactive constituents of ginseng. The use of cell and organ culture processes has been sought as a potential alternative for the efficient mass production of ginseng raw material. Various bioprocessing strategies have been developed to date. Cells and adventitious roots have been cultured in large-scale bioreactors and various strategies have been developed accordingly for the enhancement of biomass and ginsenoside accumulation. This review highlights the recent progress in the cultivation of ginseng cell and organ cultures for the production of ginsenosides from bioreactor cultures. In addition, the metabolism and biochemistry of ginsenoside biosynthesis, genomic and proteomic studies in ginseng, metabolic engineering, biosafety, toxicological evaluation, and efficacy assessment of ginseng raw material are also summarized and thoroughly discussed.

Keywords Bioreactor culture · Genomics · Ginseng · *Panax ginseng* · Saponins · Secondary metabolites

H. N. Murthy · S.-Y. Park · K.-Y. Paek (✉)
Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, Cheongju 361-763, Republic of Korea
e-mail: paekky@chungbuk.ac.kr

H. N. Murthy (✉)
Department of Botany, Karnatak University, Dharwad 580003, India
e-mail: hnmurthy60@gmail.com

M. I. Georgiev
The Stephan Angeloff Institute of Microbiology, Bulgarian Academy of Sciences, 139 Ruski Blvd., 4000 Plovdiv, Bulgaria

Y.-S. Kim
Natural Resources Research Institute, R & D Headquarters, Korea Ginseng Corporation, 22, Gajeong-ro, Shinseong-dong, Yuseong-gu Daejon 305-805, Republic of Korea

C.-S. Jeong
Osong Medical Innovation Foundation, Clinical Drug Manufacturing Center, Chungbuk 363-951, Republic of Korea

S.-J. Kim
Gwacheon National Science Museum, Ministry of Science, ICT and Future Planning, 110 Sanghabeol-ro, Gwacheon-Si, Gyeonggi-do 427-060, Republic of Korea

Introduction

Panax ginseng C.A. Meyer (Araliaceae), commonly known as ginseng, is one of the most important medicinal plant species. It is widely used as a tonic and an adaptogenic agent in oriental countries such as China, Japan, and Korea. It is also popular as a nutraceutical or as a functional food in rest of the world, especially in North America and Europe. "*Panax*" is derived from the Greek word "Panacea", which means "cure all diseases". It is used for longevity, as well as for improving physical strength and resistance (Cheng et al. 2005; Xie et al. 2011). The principal active constituents of ginseng are triterpenoid saponins, known as ginsenosides. Ginsenosides are secondary metabolites that are classified into three groups based on their structure—the Rb group (panaxadiols, including Rb1, Rb2, Rc, Rd, and so forth), the Rg group (panaxatriols, including Rg1, Re, Rf, Rg2, and so forth), and the Ro group (oleanolic acid) (Fig. 1; Park et al. 2005). Other than saponins, ginseng roots also contain biophenols,

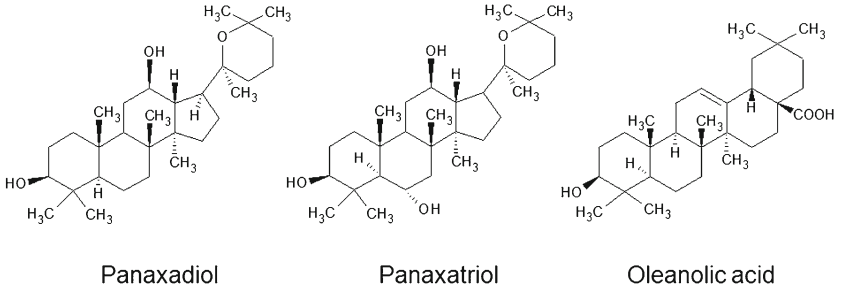

Fig. 1 Structures of panaxadiol, panaxatriol, and oleanane ginsenosides

polyacetylenes, sesquiterpenes, polysaccharides, peptidoglycans, fatty acids, and vitamins. The pharmacological effects of ginseng have been demonstrated in cancer; diabetes mellitus; cardiovascular, immune, and nervous disorders; including their anti-stress and anti-oxidant activities (Park et al. 2005). Due to these pharmacological effects, ginseng has become a popular tonic and a health food in oriental, as well as in Western countries. Recently, the products of ginseng have also been used in the cosmetic industry and in health beverage preparations (Lee 2005; Lee et al. 2010; Chung et al. 2011).

Ginseng plant material collected from its natural habitat is highly expensive and occurs very rarely in nature. Ginseng is also cultivated in the fields; however, it usually takes 5–7 years from seedling to final harvesting stage of roots, during which close attention is needed as its growth is influenced by various conditions such as soil, climate, pathogens, and pests. In addition, its fresh roots are not available during all seasons (Paek et al. 2009; Sivakumar et al. 2005b). Current advances in plant biotechnology have made it possible to culture plant cells for the production of valuable metabolites (Georgiev et al. 2013). Ginsenoside production through ginseng cell cultures has been successfully achieved and extensively reported in the literature (Akalezi et al. 1999; Furuya et al. 1983b; Liu and Zhong 1998; Zhong 1998). However, the large-scale culture of plant cells for the commercial production of useful secondary metabolites remains challenging due to its relative low productivity and biochemical instability of plant cells grown in vitro (Payne et al. 1991; Wu and Zhong 1999). Thus, differentiated organ cultures seem to be promising for the production of useful secondary metabolites (Murthy et al. 2014). In addition, genetically transformed root cultures (=hairy rots) have been recommended based on their merit of rapid growth characteristics (Yu et al. 2005b; Georgiev et al. 2012). Total extracts obtained from some hairy root cultures, however, contain opine-like compounds, which have been shown to be potentially harmful to mammalian cells (Yoshikawa and Furuya 1987). Therefore, ginseng adventitious root cultures represent an excellent alternative as its growth is relatively fast and its ginsenoside production is relatively stable, without any potential harmful molecules (Paek et al. 2009). Several reports on ginseng cell and adventitious root cultures have focus on increasing biomass growth and ginsenoside productivity by manipulating the culture medium and culture environment. It is also possible to boost the volumetric yield of ginsenosides by applying various strategies such as elicitation, application of suitable bioreactor design, and bioprocess technologies. This review summarizes the methods of in vitro culturing of ginseng cells/adventitious roots and the mass production of ginsenosides from bioreactor cultures. In addition, reports on the metabolism and biochemistry of ginsenoside biosynthesis, genomic studies, metabolic engineering approaches, biosafety, toxicological evaluations, as well as efficacy tests of ginseng biomass are thoroughly discussed.

Establishment of cell suspension and adventitious root cultures: from field to bench

Adventitious roots were induced from the root explants of 100 years old mountain ginseng (*P. ginseng* C.A. Meyer; Fig. 2a) on Murashige and Skoog (MS 1962) medium, supplemented with growth regulators (Son et al. 1999; Yu et al. 2000). The root segments developed calli on MS medium supplemented with 4.53 μM 2,4-dichlorophenoxy acetic acid (2,4-D), 0.46 μM kinetin, and 3 % sucrose (Fig. 2b). Furthermore, adventitious roots were developed from calli masses cultured on MS medium supplemented with 19.68 μM indole-3-butyric acid (IBA) and 3 % sucrose (Fig. 2c). Furuya et al. (1983a) found that 2,4-D was essential for callus induction in ginseng, whereas IBA was determined as the most suitable auxin for adventitious roots induction. The actively growing adventitious root clones were further selected (Fig. 2d) and cultured in MS liquid medium supplemented with 19.68 μM IBA and 3 % sucrose in 1-L capacity bottle bioreactors containing 600 mL of medium until these reached a certain biomass (Fig. 2e). Finally, the roots were transferred to 5-L airlift bioreactors containing 3 L of MS medium as previously mentioned and maintained once every 6 weeks (Fig. 2f).

Optimization of medium and physical parameters

Optimization of culture medium and key physical factors appears as an essential and effective technique to enhance biomass accumulation and secondary metabolite production

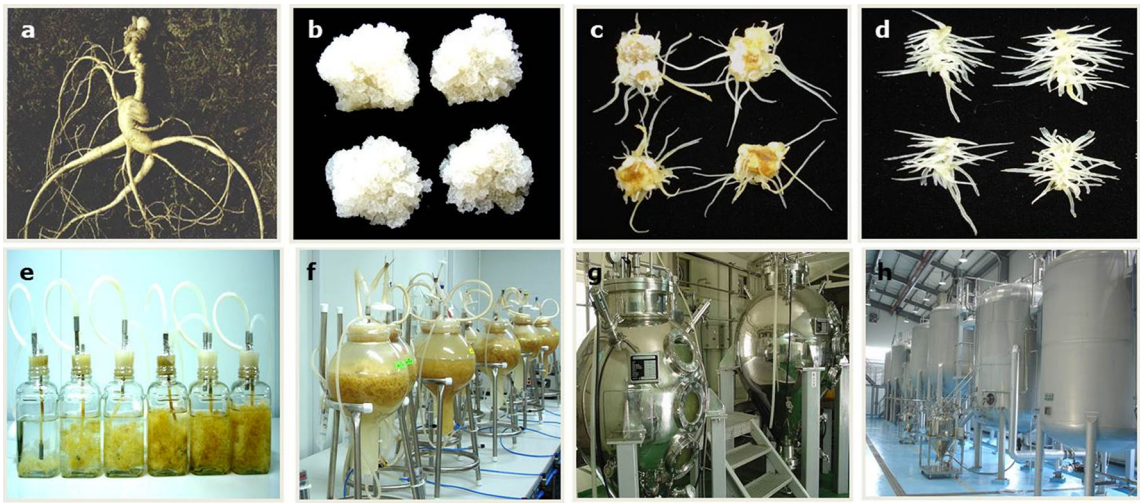

Fig. 2 Flow diagram showing the induction and cultivation of adventitious roots: 100-year-old mountain ginseng (*Panax ginseng* C.A. Meyer) root (**a**), callus (**b**), induction of adventitious roots from callus (**c**), selected adventitious root clones (**d**), adventitious roots in 1-L bioreactors (**e**), adventitious roots in 5-L bioreactors (**f**), 1,000-L bioreactors (**g**), and 10,000-L bioreactors

(Georgiev and Weber 2014; Murthy et al. 2014). The suitability of medium, salt strength, growth regulator type, combination and concentration, type and concentration of carbohydrate, nitrogen source and its concentration, physical conditions such as light and temperature, agitation and aeration to ginseng cell, and adventitious root cultures have been investigated systematically by different research groups (Table 1).

The effect of medium and salt strength

The adventitious roots were cultured on MS medium of different salt strengths to test their efficiency for biomass growth and ginsenoside production. Different salt strengths of MS medium were tested and the MS medium of 0.75× salt strength showed maximum biomass and growth, whereas the higher ginsenoside production was achieved with 0.5× salt strength MS medium (Sivakumar et al. 2005a). MS medium with 2.0× salt strength showed very slow growth, which affected biomass accumulation as well as overall ginsenoside productivity (Sivakumar et al. 2005a). However, Son et al. (1999) used Woody plant medium (WPM; Lloyd and McCown 1981) for biomass growth and metabolite productivity. The selection of different strains of cells or adventitious roots might have led to such variation. Therefore, selection of suitable cell/adventitious root clones and selection of suitable medium and salt strength are of primary importance for the establishment cell or organ cultures.

The effect of growth regulators

Plant growth regulators are one of the key factors influencing biomass growth and secondary metabolite production. In suspension cultures of *Panax notoginseng* and *Panax ginseng*, Zhong (1998) demonstrated the significant influence of individual growth regulator levels as well as their combinations on cell growth and ginsenoside accumulation. In a large-scale culture process (630-L stirred tank bioreactor) reported by Shamakov et al. (1991), α-naphthalene acetic acid (NAA) and kinetin were used as growth regulators instead of 2,4-D. The effect of two exogenous auxins, IBA (5, 12, 25, 37, and 49 μM), and NAA (5, 11, 16, 25, and 27 μM), were tested by Jeong et al. (2009) for the multiplication of adventitious roots, and they have shown profuse development of lateral roots from the inoculated roots in the medium supplemented with IBA and NAA, whereas no development of lateral roots in the auxin-free medium. The roots formed on IBA-supplemented medium were slender and elongated, whereas the lateral roots formed in NAA-supplemented medium were shorter and thicker. Among the different concentrations of IBA tested, 25 μM was suitable for lateral root development; each root explant developed 31.4 lateral roots, which was the highest root biomass after 40 days of culture (10.2 g/L dry weight).

The effect of inoculum density

Inoculum density is one of the important factors that influences biomass and metabolite accumulation. There is a critical minimal inoculum density/size, below which cell or root growth will normally fail. In the case of ginseng adventitious roots, various densities of inoculum were tested, i.e., 2.5, 5.0, 7.5, and 10.0 g fresh weight (FW)/L. The results showed that 5 g/L of inoculum was suitable for biomass accumulation [10.5 g dry weight (DW)/L] and ginsenoside productivity (5.4 mg/g DW; Jeong et al. 2009).

The effect of ammonium/nitrate ratio

The effect of ammonium/nitrate ratio (NH_4^+/NO_3^-; 0.0:18.5, 7.19:18.5, 14.38:18.5, 21.57:18.5, 28.75:18.5, 14.38:0.0,

Table 1 Strategies employed for the improvement of ginseng biomass and secondary metabolites in vitro

Sl. no	Strategies/achievements	References
1	Induction of adventitious roots from mountain ginseng and selection of adventitious root clones	Son et al. (1999); Yu et al. (2000)
2	Effect of medium on adventitious root growth and ginsenoside production	Son et al. (1999); Yu et al. (2000); Sivakumar et al. (2005a, b)
3	Effect of plant growth regulators on cell and adventitious root growth and ginsenoside production	Yu et al. (2000); Sivakumar et al. (2005a, b); Jeong et al. (2009)
4	Effect of inoculum density on adventitious root growth and ginsenoside production	Jeong et al. (2009)
5	Effect of macro- and microelements in the culture medium on cell and adventitious root growth and ginsenoside production	Liu and Zhong (1998); Yu et al. (2000); Sivakumar et al. (2005a, b)
6	Effect of sucrose concentration and osmotic agents on cell and adventitious root growth and ginsenoside production	Zhang et al. (1996b); Akalezi et al. (1999); Yu et al. (2000); Sivakumar et al. (2005a, b)
7	Effect of bioreactor types on adventitious root growth and ginsenoside production	Yu et al. (2000); Kim et al. (2004a)
8	Effect of aeration rate and sparger type on adventitious root growth and ginsenoside production	Kim et al. (2005)
9	Effect of oxygen, carbon dioxide, and ethylene on cell and adventitious root growth and production of ginsenosides	Jeong et al. (2006)
10	Effect of methyl jasmonate elicitation on cell and adventitious root growth and production of ginsenosides	Yu et al. (2002); Kim et al. (2004b); Hu and Zhong (2007); Wang and Zhong (2002); Thanh et al. (2005)
11	Effect of organic germanium elicitation on adventitious root growth and production of ginsenosides	Yu et al. (2005b)
12	Effect of polyunsaturated fatty acids elicitation on adventitious root growth and production of ginsenosides	Wu et al. (2009); Dewir et al. (2010)
13	Effect of heavy metals on production of ginsenosides	Huang and Zhong (2013)
14	Effect of *N,N'*-dicyclohexylcarbodiimide (DCCD) elicitation	Huang et al. (2013)
15	Effect of medium replenishment strategy on adventitious root growth and production of ginsenosides	Jeong et al. (2008)
16	Effect of precursor feeding on adventitious root growth and production of ginsenosides	Sivakumar et al. (2006a)
17	Effect of feeding-conditioned medium	Woragidbumrung et al. (2001)
18	Effect of high-density cultures	Zhang and Zhong (1997); Zhong et al. (1999)
19	Use of stirred tank bioreactors for large-scale cell culture	Strogov et al. (1990); Shamakov et al. (1991)
20	Use of airlift bioreactors for large-scale cultures of cells and adventitious roots	Asaka et al. (1993); Paek et al. (2009)

14.38:9.4, 14.38:18.8, 14.38:26.2, and 14.38:37.6 mM) on biomass growth and ginsenoside accumulation has been studied by Sivakumar et al. (2005a) and their results showed that nitrate plays an important role in biomass increase and ginsenoside production, instead of ammonium. A low ammonium concentration combined with a high nitrate concentration was favorable for root growth, showing the largest amount of root biomass at an ammonium/nitrate ratio of 7.19:18.5. Maximum ginsenoside yield was achieved at a ratio of 0 mM ammonium to 18.5 mM nitrate. Similar experimental observations were reported with cell cultures of *P. ginseng* and *Panax notoginseng* (Ushiyama 1991; Zhang et al. 1996a), where MS medium with altered nitrogen concentrations was used for the cultivation of ginseng cells and adventitious roots.

The effect of phosphate

Phosphate is another key nutrient for plant cell and organ growth. Liu and Zhong (1998) reported that an increase in initial phosphate from 1.25 to 3.75 mM enhanced cell growth as well as ginsenoside yield in suspension cultures of *P. notoginseng* cells. Choi et al. (1994) reported that saponin content decreased with an increase in phosphate concentrations from 3.7 to 11 mM.

The effect of carbohydrate source

Carbohydrates are essential carbon and energy source for plant cell and organ cultures and it has been demonstrated that the initial sucrose concentration can affect a number of cultural parameters such as growth and yield of secondary metabolites (Zhang et al. 1996b). Choi et al. (1994) reported that the optimal concentration of sucrose for cell growth was within the range of 30–50 g/L, and that 70 g/L of sucrose inhibited cell growth, whereas the ginsenoside content showed a steady increase with elevated sucrose concentrations of up to 60 g/L. In suspension cultures of *P. notoginseng* cells, Zhang and Zhong (1997) showed that constant or intermittent

feeding of sucrose and other sugars was more effective than increasing its initial concentration. The influence of sucrose at a range of 1–9 % was tested on biomass growth and accumulation of ginsenosides in adventitious root cultures by Sivakumar et al. (2005a) and the results showed that 5 % sucrose is suitable for biomass as well as ginsenoside accumulation. Fresh and dry biomass of adventitious roots decreased with sucrose concentrations >5 %. Therefore, a 50 g/L initial sucrose concentration is generally used for cultivation of adventitious roots in bioreactors.

The effect of light and temperature

Light and temperature are the two physical factors that influence cell and organ culture growth. Previous studies have shown that the optimal temperature and light treatment of suspension cultures are essential for the accumulation of biomass and production of metabolites (Yu et al. 2005a; Zhong et al. 1991). Incubation of cultures at 20 °C is most suitable for enhancement of ginseng adventitious root biomass and ginsenoside accumulation among 10, 15, 20, 25, and 30 °C temperature regimes tested (Paek et al. 2009). Yu et al. (2000) tested the effect of light quality (red, blue, and blue plus red lights) and light intensity (photon flux) on ginseng adventitious root cultures and reported no positive effects. Therefore, ginseng adventitious root cultures are maintained in the dark and at a constant ambient temperature of 20 °C.

The effect of oxygen, carbon dioxide, and ethylene

Biomass growth and accumulation of metabolites in cultures are influenced by a gaseous composition of oxygen, carbon dioxide, and ethylene. Natural atmospheric sterilized air (N_2, 78 %; O_2, 20.8 %; Ar, 0.9 %; and CO_2, 0.03 %) was enriched with pure oxygen (30 and 40 %), carbon dioxide (2.5 and 5 %), or ethylene (20 and 20 ppm) to promote ginseng adventitious root growth and accumulation of metabolites (Jeong et al. 2006). Only the enrichment with 40 % O_2 showed enhancement of root biomass accumulation and ginsenoside production, whereas enhanced levels of CO_2 and ethylene were unfavorable to the cultures, which finally resulted in a decrease in the accumulation of ginsenosides.

Elicitation: mimicking plant defence to boost targeted metabolite yields

Elicitors are chemicals or biofactors from various sources (biological, chemical, physical, or mechanical) that can induce physiological changes in the target organism. In a broad sense, elicitors in a plant refer to chemicals from various sources that can trigger physiological and morphological responses, besides phytoalexin accumulation (Zhao et al. 2005). Elicitation has been shown to be an effective strategy for the induction of secondary metabolites in plant cell and organ cultures of ginseng (Table 1).

Jasmonates and salicylic acid are important signaling molecules that have been used as exogenous elicitors to trigger secondary metabolism in plant cell and organ cultures. Kim et al. (2004b) and Thanh et al. (2005) tested the efficacy of methyl jasmonate (MJ) in the elicitation of ginseng cell and adventitious root cultures for the synthesis of ginsenoside. The adventitious root cultures treated with 100 μM MJ showed a sevenfold enhancement in the accumulation of ginsenosides when compared to the control (untreated roots). However, MJ treatments (50–150 μM) led to a decrease in biomass accumulation. Therefore, a two-step strategy was adopted by Yu et al. (2002) and Kim et al. (2004b) for the maintenance of adventitious root cultures without elicitors for 40 days, which involved treatment of cultures with 100 μM MJ and maintaining them for another 10 days. The manipulation of ginsenoside heterogeneity has been reported in ginseng cell cultures treated jasmonates (Wang and Zhong 2002; Hu and Zhong 2007). Organic germanium, vanadate, *N,N′*-dicyclohexylcarbodiimide, and polyunsaturated fatty acid (PUFAs) have also been demonstrated as highly effective elicitors for increasing ginsenoside production (Dewir et al. 2010; Huang and Zhong 2013; Huang et al. 2013; Wu et al. 2009; Yu et al. 2005b).

The effect of precursor feeding and medium replenishment strategies

Strategies such as precursor or fresh nutrient medium feeding (fed-batch culture) have been shown by several groups (Lipsky 1992; Woragidbumrung et al. 2001; Zhang and Zhong 1997) to have a positive influence on biomass accumulation and production of secondary metabolites. Ginseng adventitious root cultures were fed with biogenic precursor squalene resulted in a positive effect on biomass and metabolite productivity (Sivakumar et al. 2006a). Medium replenishment method was employed by Jeong et al. (2008), which resulted in a 26 % increase in dry biomass (28.7 g/L) and an 8.3 % enhancement of ginsenoside content (4.92 mg/g DW). These results clearly indicate that precursor feeding and medium replenishment are useful strategies for enhancing secondary metabolite production.

Large-scale bioreactor culture: toward commercial production

In general, the basic function of a bioreactor is to provide optimum conditions for cell physiology and metabolism by regulating various environmental (chemical and physical)

factors. The main criteria for designing plant cell/organ culture bioreactors should consider adequate oxygen transfer, low shear stress, and good mixing (Georgiev et al. 2013). Stirred-tank and airlift bioreactors are most widely used for ginseng cell and adventitious root cultures. Various operation methods such as draw-fill, fed batch, and semicontinuous and continuous processes have been suggested (Lipsky 1992; Zhang and Zhong 1997). High-density cell cultures drive the production of metabolites to a higher rate compared to the low-density cultures (Strogov et al. 1990; Zhang and Zhong 2004; Zhong et al. 1999).

Asaka et al. (1993) used an airlift bioreactor and a stirred-tank reactor with two different types of impellers, a flat-blade turbine and a paddle impeller, for the growth of embryogenic tissues and observed a threefold higher biomass and elevated saponin productivity in the airlift bioreactors compared to the stirred-tank reactors. For adventitious root cultures of ginseng, airlift bioreactors have been proven to be suitable for both biomass and metabolite production (Paek et al. 2009). Among various configurations of airlift bioreactors such as bulb, cone, cylinder, and balloon type, the balloon type of airlift bioreactors was found suitable for both accumulation of ginseng biomass and secondary metabolites (Kim et al. 2004a). The diameter and the pore size of the sparger, as well as the aeration rate were found to influence ginsenoside productivity (Kim et al. 2005).

The design of a suitable bioreactor and the selection of an appropriate cultivation method are necessary to achieve a significant enhancement in the productivity of secondary metabolites at a commercial scale. Hence, a commercial-scale bubble bioreactor of size 1,000 (Fig. 2g) and 10,000 L (Fig. 2h) (commercial bioreactors were manufactured by Kihyung Plant Co., Ltd., Suwon, South Korea) were tested and used by CBN Biotech Company, South Korea for the production of ginseng adventitious root biomass using MS medium supplemented with 5 mg/L IBA and 50 g/L sucrose. The root growth pattern was similar to that of small-scale cultures and 850 kg of fresh and 85.4 kg dry biomass could be produced in each batch cycle of 45 days. With the operation of four 10,000-L bioreactors, CBN Biotech Company is producing ~35 t of ginseng adventitious roots per year, which is a spectacular example of the application of plant biotechnology to meet the needs of the pharmaceutical, food, and cosmetic industry.

Metabolism and biochemistry of ginsenoside biosynthesis

Ginsenosides are the major components of ginseng (Fig. 1). Ginsenosides possess a dammarane triterpenoidal skeleton with a modified side chain C-20 (Huang 1999; Park et al. 2005). They differ from one another by the type of sugar moieties, as well as their number and site of attachment (Park et al. 2005). Among the saponins, the genuine sapogenins protopanaxadiol and protopanaxatriol have been identified as dammar-24-ene-3β,12β,20(*S*)-triol and dammar-24-ene-3β,6α12β,20(*S*)-tetrol, respectively (Shibata 2001). Triterpene and sterol biosynthesis involve a common pathway using C-5 isoprenoids. Mevalonate is the preferential precursor for sterol and triterpene biosynthesis (Kuzuyama 2002; Fig. 3). The enzyme squalene synthase catalyses the first step from the central isoprenoid pathway toward sterol and triterpenoid biosynthesis (Abe et al. 1993). Both polysterols and triterpenes in plants are synthesized from the product of cyclization of 2,3-oxidosqualene. This step in ginsenoside synthesis involves the cyclization of 2,3-oxidosqualene to oleanane and a dammarene-type triterpene skeleton. Kushiro et al. (1998) isolated several genes encoding oxidosqualene cyclase, and these genes were shown to be highly homologous to each other.

Cytochrome P450 and glycosyltransferase enzymes are involved in a further step of ginsenoside biosynthesis. A cytochrome P450 member hydroxylates the C-6 position of protopanaxadiol, producing the protopanaxatriol, and the two latter compounds form the backbone of ginsenosides. The biosynthesis of ginsenosides from triterpene aglycone involves glycosylation at the C-3 and C-20 hydroxyl positions on the skeleton for the protopanaxadiol type and the C-6 and C-20 positions for the protopanaxatriol type ginsenoside (Choi et al. 2005).

Genomics and proteomics studies in ginseng

Construction of a bacterial artificial chromosome (BAC) library and sequence analyses of expressed sequence tags (ESTs) has been initiated in ginseng with the objective of elucidating the ginseng genome. Hong et al. (2004) reported that the ginseng BAC library consists of 106,368 clones with an average size of 98.61 kb, accounting for 3.34 genome coverage. Sequencing of 2,167 BAC clones generated 2,492 BAC end-sequences with an average length of 400 bp. BLAST analysis and motif searches revealed that 10.2, 20.9, and 3.8 % of the BAC end-sequences contained protein-coding regions, transposable elements, and microsatellites, respectively.

ESTs provide a valuable tool for the identification of genes in *P. ginseng*. Approximately 26,000 ESTs of *P. ginseng* sequences are currently available at http://plant.pdrc.re.kr:7777/index.html. Eight cDNA libraries for EST sequencing were constructed from different organs, including taproot, rhizome, developing seed, in vitro-grown seedlings, and soil-grown seedlings. Jung et al. (2003) sequenced 11,636 ESTs from five ginseng libraries to create a gene resource for the biosynthesis of ginsenosides. Only 59 % of the ginseng ESTs exhibited significant homology to the

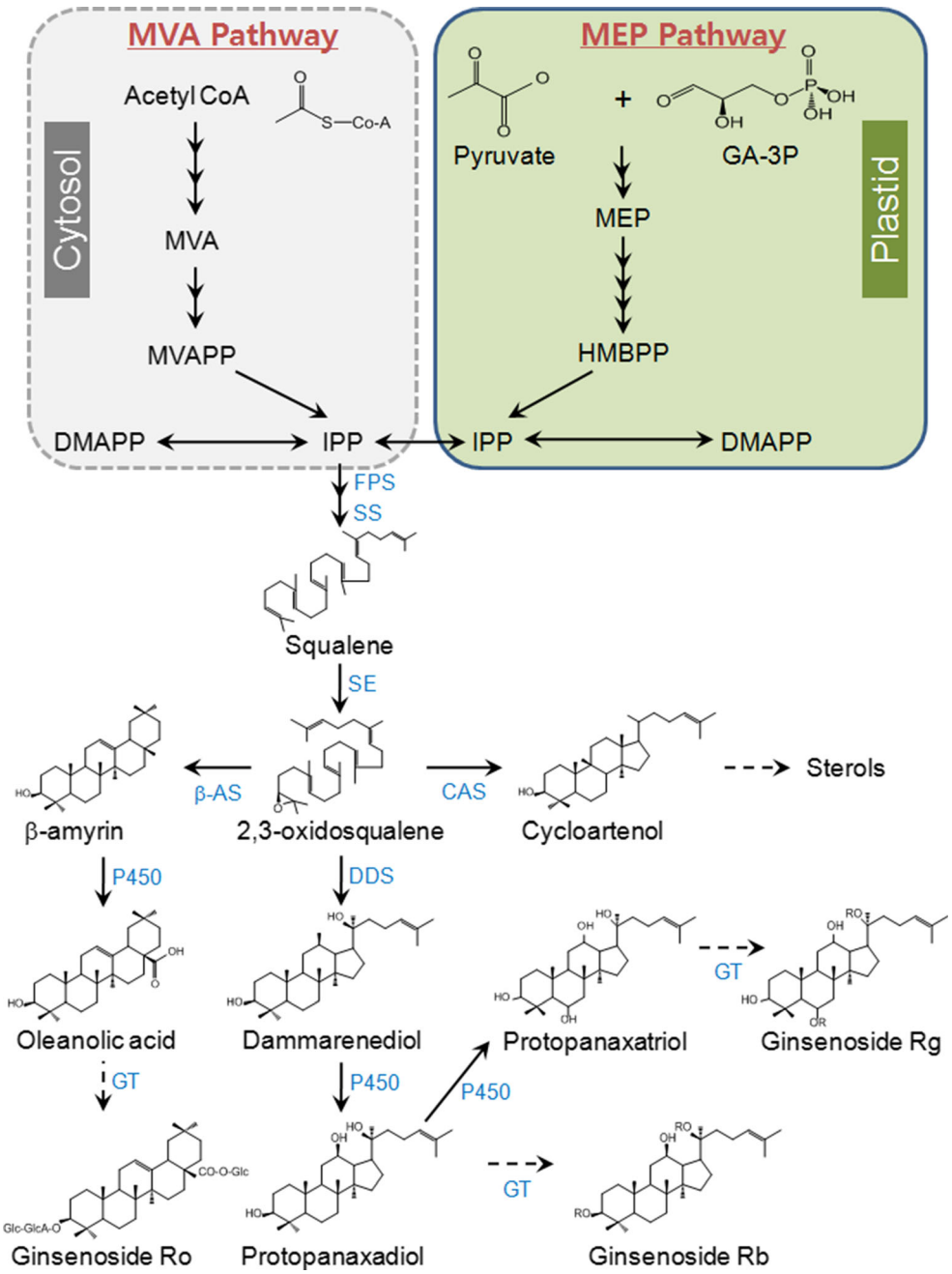

Fig. 3 The putative biosynthetic pathway of ginsenosides. *MVA* mevalonate, *MVAPP* mevalonate-5-diphosphate, *DMAPP* dimethylallyl diphosphate, *IPP* isopentenyl diphosphate, *MEP* methylerythritol phosphate, *HMBPP* (E)-4-hydroxy-3-methyl-but-2-enyl diphosphate, *FPS* farnesyl diphosphate synthase, *SS* squalene synthase, *SE* squalene epoxidase, *DDS* dammarenediol synthase, *β-AS* β-amyrin synthase, *CAS* cycloartenol synthase, *P450* cytochrome P450, *GT* glucosyltransferase. The *dotted line* indicates the putative pathway

previously known polypeptide sequences. Stress- and pathogen-response proteins were most abundant in the roots of 4-year-old ginseng. The above-mentioned authors identified 4 oxidosqualene cyclase candidates involved in the cyclization reaction of 2,3-oxidosqualene, as well as 9 cytochrome P450 and 12 glycosyltransferase candidates that might be involved in the modification of the triterpene backbone.

Choi et al. (2005) previously used hairy roots and adventitious roots of ginseng treated with MJ as a system to trigger the genes involved in ginsenoside biosynthesis. They generated 3,134 ESTs from MJ-treated ginseng hairy roots and these were assembled into 370 clusters and 1,680 singletons. Genes that showed high expression were those that encode for proteins involved in fatty acid desaturation, defence response, and secondary metabolite biosynthesis. Further analysis revealed that a number of genes, including those encoding oxidosqualene cyclase (OSC), cytochrome P450, and glycosyltransferase were involved in ginsenoside biosynthesis. Xu et al. (2005) reported that fungal elicitors induced singlet oxygen generation, subsequently leading to ethylene release and increased ginsenoside synthesis. Han et al. (2006) reported that the ectopic expression of the dammarenediol synthase gene (*DDS*) in a yeast mutant (*erg7*) lacking lanosterol synthase resulted in the production of dammarenediol and hydroxydammarenone.

Furthermore, Kim et al. (2010) isolated three new *P. ginseng* squalene synthase genes (*PgSS1*, *PgSS2*, and *PgSS3*) that enhanced *P. ginseng* sterol and triterpene saponin biosynthesis. Han et al. (2011) isolated nine putative full cytochrome P450 (CYP) sequences from the ESTs of MJ-treated adventitious roots. An in vitro enzymatic assay revealed that CYPA16A47 catalyzed the oxidation of dammarenediol-II to produce protopanaxadiol. Recently, Han et al. (2012) isolated two additional CYP716A subfamily genes (*CYP716A52v2* and *CYP716A53v2*) and determined that the gene product of *CYP716A53v2* is protopanaxadiol 6-hydoxylase, which catalyses the formation of protopanaxatriol from protopanaxadiol during ginsenoside biosynthesis. More recently, Han et al. (2013) have shown that *CYP716A52v2* encodes a β-amyrin 28-oxidase, which modifies β-amyrin into oleanolic acid, a precursor of an oleanane-type saponin (mainly ginsenoside Ro) in *P. ginseng*. Li et al. (2013) sequenced the transcriptome of the roots, stems, leaves, and flowers of 4-year-old *P. ginseng* plants, resulting in the identification of 14 potential ginseng microRNAs (miRNAs) and 100 of their potential target genes. In addition, 13,044 simple sequence repeats (SSRs) were identified from ginseng unigenes.

Additionally, Kim and Lee (2004) sequenced the entire chloroplast genome of *P. ginseng*, which they reported as a double-stranded DNA consisting of 156,318 bp. One-hundred and fourteen genes were identified in their analysis, of which 75 were peptide-encoding genes, 30 were tRNA genes, 4 were rRNA genes, and 5 were conserved open-reading frames.

A number of proteomic studies have been conducted in *P. ginseng* to gain a better understanding of the ginsenoside biosynthetic pathway and to increase the production of ginseng plants. The lack of genomic DNA sequence information on *P. ginseng* constitutes a major bottleneck with regard to the identification of proteins in proteomics studies (Nam et al. 2005). Nevertheless, Nam et al. (2003) analyzed the proteins expressed in ginseng leaves that were exposed to 0–4 h of light. These proteins include three cytosolic small heat-shock proteins, cytosolic ascorbate peroxidase, and putative major latex-like proteins, which were all upregulated by light. The other three proteins, Rieske Fe/s protein, putative 3-beta hydroxysteroid dehydrogenase/isomerase-like protein, oxygen-evolving enhancer-like proteins, were downregulated. Miskell et al. (2002) also isolated a few photosynthesis-related proteins in *P. quinquefolius*.

Metabolic engineering attempts in ginseng

Lee et al. (2004) investigated the role of squalene synthase in the biosynthesis of phytosterols and triterpenoids and cloned the *PgSS1* gene to ginseng via *Agrobacterium*-mediated transformation. Transgenic *P. ginseng* plants harboring *PgSS1* exhibited a remarkable increase in the production of β-sitosterol, stigmasterol, campesterol, and ginsenosides. The overexpression of the *PgSS1* gene in *P. ginseng* roots was also accompanied by the upregulation of downstream genes such as squalene epoxidase, β-amyrin synthase, and cycloartenol synthase. Therefore, cloning of *P. ginseng* with genes such as squalene synthase (*PgSS1*), squalene epoxidase genes (*PgSQE1* and *PgSQE2*), and cytochrome P450 subfamily genes (*CYP716A52v2* and *CYP716A53v2*) would be a useful strategy toward the enhancement of ginsenosides production.

Processing of in vitro-produced ginseng biomass and extraction of ginsenosides

Drying of ginseng raw material is one of the most important processes in ginsenoside extraction. This process involves the removal of moisture, which is a critical threshold that allows long-term storage of the product, while maintaining its quality. Kim et al. (2008) developed the processing technology for ginseng adventitious roots, which consists of forced air-drying, and far infrared and freeze-drying methods (Table 2). They found that forced air-drying at 50 °C for 10 h was suitable for processing of ginseng adventitious roots and the dried roots possessed optimal concentrations of ginsenosides. Kim et al. (2007) reported a method for ginsenoside extraction from the ginseng adventitious roots; they conducted heat reflux solvent extraction, as well as ultrasonic and microwave-assisted extraction methods. Moreover, the authors reported that the heat reflux solvent extraction method was a superior technique compared to the ultrasonic and microwave-assisted extraction methods.

Toxicological evaluation and efficacy test of ginseng biomass produced in vitro

Toxicological and biosafety evaluation of the medicinal raw material is an essential step prior to its human consumption. Toxicological and biosafety evaluation of ginseng

Table 2 Processing, extraction of bioactive compounds and toxicological evaluation of ginseng biomass obtained from in vitro culture

Sl. no	Drying/processing, extraction and toxicity evaluation	References
1	Effect of processing methods on the concentrations of bioactive components of ginseng adventitious roots	Kim et al. (2008)
2	Effect of extraction methods on the concentrations of bioactive components of ginseng adventitious roots	Kim et al. (2007)
3	Safety and toxicity evaluation of ginseng adventitious roots	Sivakumar et al. (2006b)

Table 3 Efficacy tests of ginseng biomass obtained from in vitro culture

Sl. no	Types of efficacy tests	Reference
1	As a fertility agent in treating spermatogenic disorders	Park et al. (2006)
2	Inhibition of platelet aggregation activity and activation in human whole blood	Jeon et al. (2008); Lee et al. (2011)
3	Treatment of erectile dysfunction	Kim et al. (2009)
4	Treatment of hyperlipidemia	Lee et al. (2003)
5	Antifibrotic activity	Lim et al. (2007)
6	Anti-oxidant activity	Ali et al. (2006); Lim et al. (2007)
7	Stimulation of immune cells and inhibition of cancer cell proliferation	Oh et al. (2006)
8	Augmentation of peripheral blood flow	Lee et al. (2011)
9	Inhibition effect of L-DOPA oxides activity of tyrosinase (skin whitening activity)	Xu et al. (2008)

adventitious roots was carried out by Sivakumar et al. (2006b) (Table 2) and they referred to the ginseng adventitious root biomass as the tissue cultured mountain ginseng adventitious roots (TCMGARs). Mutagenicity tests, including reverse mutation test in *Salmonella typhimurium* strains TA 93, TA 100, TA 1535, and TA 1537 and the *Escherichia coli* strain WP2*uvr*A, did not show significant mutagenicity. A chromosomal aberration test using Chinese hamster lung (CHL) cultured cells and a rat micronucleus test also proved that TCMGARs are nontoxic. Furthermore, 13 weeks of repeated dose toxicity of TCMGAR oral doses ranging from 300 to 900 mg/kg did not result in any mortality or significant changes in the general behavior and gross appearance of internal organs of experimental rats and Beagle dogs. Histopathological examinations of various organs and hematological tests revealed no differences between the control and the TCMGAR-fed animals. These results confirmed the biosafety of TCMGARs and based on such evaluation, the Korean Food and Drug Administration (KFDA), ISO (9001/2000), and the United States Food and Drug Administration (USFDA) have approved (2030950, dt. 06/07/2002) the commercial production of ginseng adventitious roots and their products.

Pharmacological effects of ginseng adventitious roots have been carried out by various research groups and have shown that in vitro-raised ginseng biomass (adventitious roots) is as safe as that of natural ginseng roots (Table 3). Ginseng adventitious roots possess higher antioxidant activities than cultivated ginseng (Ali et al. 2006). The levels of polyphenolics were also highest with ginseng adventitious roots compared to that of cultivated ginseng, red ginseng, and mountain ginseng, and higher levels of antioxidant activities and polyphenolics reveal that adventitious roots are highly suitable for pharmaceutical, nutraceutical, and cosmetic industries.

Recent findings have demonstrated that ginseng adventitious root extracts and products might be useful in the prevention of spermatogenic disorders (Park et al. 2006), erectile dysfunction (Kim et al. 2006 and 2009), inhibition of platelet aggregation and thrombus formation (Jeon et al. 2008; Lee et al. 2011), symptoms of hyperlipidemia (Lee et al. 2003), antifibrotic activities (Lim et al. 2007). In addition, ginseng adventitious root extracts stimulate immune cells and possess

Fig. 4 Ginseng-based products prepared from ginseng adventitious roots. **a** Ginseng-based cosmetics, **b** ginseng wine, **c** ginseng soap, **d** ginseng syrup, **e** and **d** ginseng tonic

antineoplastic activities (Oh et al. 2006) besides imparting skin whitening effects as shown by novel polyacetylenes accumulated in ginseng adventitious roots (Xu et al. 2008). After successful evaluation of its biosafety, toxicological, and therapeutic values, a wide range of ginseng products such as ginseng powder, syrup, wine, soap, and ginseng-based cosmetics is now available in the Korean market (Fig. 4). Ginseng health products have also gained the status of functional food and several Korean companies currently promote these products.

Conclusions and further perspectives

Ginseng cell and organ culture technologies have been developed for its large-scale production and commercialization. However, certain areas of the production process require further improvement, particularly relating to the cell and organ culture technology and bioprocessing methods. Recently, several genes that are involved in the ginsenoside biosynthetic pathway have been identified and cloned; nevertheless, the entire biosynthetic pathways and the associated genes have not yet been revealed. There is also a need for further extensive research in the area of genomics and proteomics of ginseng. Cloning and overexpression of well-recognized genes in ginseng through plant transformation and metabolic engineering is another area that has yet to be explored. Ginseng natural cultivation is hampered by its photosensitivity, as well as the occurrence of fungal and bacterial diseases. Thus, exploring genes that confer resistance to light, fungi, and bacteria and cloning these into ginseng are attractive areas of ginseng biotechnology.

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