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Review

Ginseng metabolic engineering: Regulation of genes related to ginsenoside biosynthesis

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Panax ginseng **is one of the most famous and widely used medicinal plants. It is generally believed that ginsenosides, tetracyclic triterpenoids, are mainly responsible for the pharmacological activities of ginseng. The genes coding for enzymes of biochemical pathways involved in triterpene biosynthesis are of considerable interest in the area of ginseng biotechnology. Upregulation of phytosterol and triterpene production by metabolic engineering of *P. ginseng* is an attractive strategy to achieve a higher medicinal value. A more detailed understanding of genes involved in saponin biosynthesis could facilitate the genetic modification of plants with altered or novel saponin content. Here the recent advances on the ginseng biotechnology particularly on the identification and characterization of genes involved in ginsenoside biosynthesis pathway were introduced.**

Key words: Expressed sequence tags, ginsenoside, *Panax ginseng*, secondary metabolite, triterpene.

INTRODUCTION

Ginseng (Panax ginseng C.A. Meyer) is a perennial herbaceous plant belonging to the family Araliaceae. Its root has been used since ancient times as an important oriental medicine that provides resistance to stress, disease and exhaustion (Sticher, 1998). The pharmaceutical activities of ginseng have been confirmed by many clinical and laboratory researches, thus the medicinal values became widely recognized in the world (Vogler et al., 1999). A variety of ginseng products are newly developed and increasingly used to promote the quality of life (Ellis and Reddy, 2002; Coleman et al., 2003). The ginseng products contain various useful components such as ginsenosides (triterpene saponins), polyacetylenes, polyphenolic compounds and acidic polysaccharides. Of these components, ginsenosides are considered to be the main active compounds accumulated in roots of ginseng. From a number of in vivo and in vitro tests, the ginsenosides have proven to take effects on immune system modulation, anti-stress activity, anticancer and anti-diabetic activities (Vogler et al., 1999; Shibata, 2001; Yun, 2001; Dey et al., 2003; quinquefolius and P. vietnamensis (Shibata, 2001). The cultivation of ginseng requires a minimum of 4 years before roots can be harvested. Ginseng should be cultivated under special conditions, where direct sunlight is blocked. Recurrent cultivation of ginseng in the same field is impossible. Therefore, farmers must prepare new ginseng fields for continuous cultivation. Moreover, various kinds of disease pose serious problems to ginseng cultivation. Agrochemical treatment to prevent disease is a serious problem for the international ginseng trade. Therefore, several researchers developed novel strategies using biotechnology techniques which contain cell cultures (Asaka et al., 1993; Furuya et al., 1994; Wu and Zhong, 1999), hairy- (Yoshikawa and Furuya, 1987; Yu et al., 2000; Jeong et al., 2003) and adventitious-root (Kevers et al., 1999; Son et al., 1999; Yu et al., 2002) cultures and bioreactor cultures (Choi et al., 2000; Kim et al., 2004). These techniques made mass production without the above problems possible. However, low production of ginsenosides in culture systems still remains to be solved (Bae et al., 2006; Kim et al., 2007). Therefore, a fundamental approach to optimize the production of ginsenoside by genetic regulation is nece-

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ssary. Metabolic engineering applications would confer solutions on the improvement of the production of ginsenosides (Lee et al., 2004; Han et al., 2006; Kim et al., 2009) and the manipulation of ginsenoside heterogeneity (Wang and Zhong, 2000; Hu and Zhong, 2007). In this review, the recent advances and perspectives in the field of ginseng metabolic engineering for adequate ginsenoside production is discussed.

GINSENOSIDE BIOSYNTHESIS

A crude ginseng root (*P. ginseng* C.A. Meyer) contains at least eight different ginsenosides which are referred to as ginsenosides Rb1, Rb2, Rc, Rd, Re, Rf, Rg1 and Ro (Shibata, 2001). Ginsenoside backbones are synthesized via the isoprenoid pathways where squalene acts as precursor. The squalene is synthesized by a series of reactions with geranyl diphosphate synthase (GPS), farnesylpyrophosphate synthase (FPS) and squalene synthase (SS) through mevalonate pathway (Kuzuyama, 2002). Subsequent reactions with squalene epoxidase yielded 2,3-oxidosqualene. The cyclization of 2,3-oxidosqualene into dammmarenediol and β- amyrin is catalyzed by oxidosqualene cyclases (OSCs), including dammarenediol-II synthase (PNA or DDS) and β-amyrin synthase (PNY). The two products are used as precursors to yield dammarene- (Rb1, Rb2, Rc, Rd, Re, Rf and Rg1) and oleanane-type (Ro) triterpene skeletons, respectively. These several genes associated with triterpene biosynthesis were previously cloned and characterized by several research groups (Kushiro et al., 1997; Kushiro et al., 1998; Lee et al., 2004; Han et al., 2006; Tansakul et al., 2006; Bang et al., 2009). However, there is no genetic information related in the next steps in ginseng, possibly hydroxylation (Shibuya et al., 2006) or glycosylation (Kushiro et al., 1997; Haralampidis et al., 2001; Choi et al., 2005). Recently, protopanaxatriol has been successfully biotransformed from protopanaxadiol using microsomal proteins evidencing protopanaxadiol 6-hydrosylase (P6H) activity (Yue et al., 2008).

IDENTIFICATION OF CANDIDATE GENES BY EXPRESSED SEQUENCE TAGS (ESTs)

Expressed sequence tags (ESTs) provide a valuable tool to identify the genes related to biosynthesis of ginsenosides in *P. ginseng*. Currently, approximately 26,000 ESTs of *P. ginseng* sequences are available at http://plant/pdrc.re.kr:7777/index.html. Eight cDNA libraries for EST sequencing were constructed from different organs, including the taproot, rhizome, developing seed, *in vitro*-cultured seedlings and soil-grown seedling shoots. Jung et al. (2003) sequenced 11,636 ESTs from five ginseng libraries in order to create a gene resource for biosynthesis of ginsenosides. Only 59% of the ginseng ESTs exhibited significant homology to previously known polypeptide sequences. Stress and pathogen response proteins were most abundant in 4-year-old ginseng roots. They identified four OSC candidates involved in the cyclization reaction of 2,3-oxidosqualene, nine cytochrome P450 and 12 glycosyltransferase candidates, which may be involved in the modification of the triterpene backbone (Figure 1).

Methyl jasmonate (MeJA) treatment can increase the production of plant secondary metabolites, including ginsenosides. To create a ginseng gene resource that contains the genes for the biosynthesis of secondary metabolites, including ginsenosides. Choi et al. (2005) generated 3,134 ESTs from MeJA-treated ginseng hairy roots. All ESTs have been submitted to the dbEST and GeneBank databases (Accession Nos. CN845540-CN848674). These ESTs were assembled into 370 clusters and 1,680 singletons. Of these ESTs, a number of genes including OSCs, cytochrome P450 and glycosyltransferase may be involved in ginsenoside biosynthesis. Recently, 4,226 ESTs from MeJA-treated adventitious roots of *P. ginseng* was sequenced. The annotated EST database was assembled into 532 clusters and 2,434 singletons. The most abundant ESTs encoded protein with binding function (36%) and enzymes involved in metabolism (15%). Through further analysis of the EST data, 8 cytochrome P450 hydroxylase and 5 glycosyltransferase that have not yet been characterized in ginseng plant was newly identified.

CHARACTERIZATION OF GENES RELATED TO GINSENOSIDE BIOSYNTHESIS

Squalene synthase

Squalene synthase catalyzes the first enzymatic step from the central isoprenoid pathway toward sterol and triterpenoid biosynthesis (Abe et al., 1993). Lee et al. (2004) characterized the cloned squalene synthase (*PgSS1*) from *P. ginseng*. *PgSS1* mRNA was ubiquitously accumulated in the various plant tissues, but higher in shoot apex and root. The transcripts level of *PgSS1* mRNA was markedly increased in the adventitious roots during 12 to 76 h period after MeJA treatment, which resulted in concurrent elevation of triterpene content, but suppression in the biosynthesis of phytosterols. Overexpression of *PgSS1* gene in adventitious roots of transgenic *P. ginseng* was followed by the up-regulation of the down stream genes, such as squalene epoxidase, β-amyrin synthase and cycloartenol synthase. Unlike MeJA treatment onto adventitious roots, enhanced activety of PgSS1 enzyme resulted in remarkable increase of phytosterol (β-sitosterol, stigmasterol and campesterol) as well as triterpene saponins (ginsenosides) content. In this work, Lee et al. (2004) suggested that *PgSS1* of *P. ginseng* is a key regulatory enzyme not only for phyto-

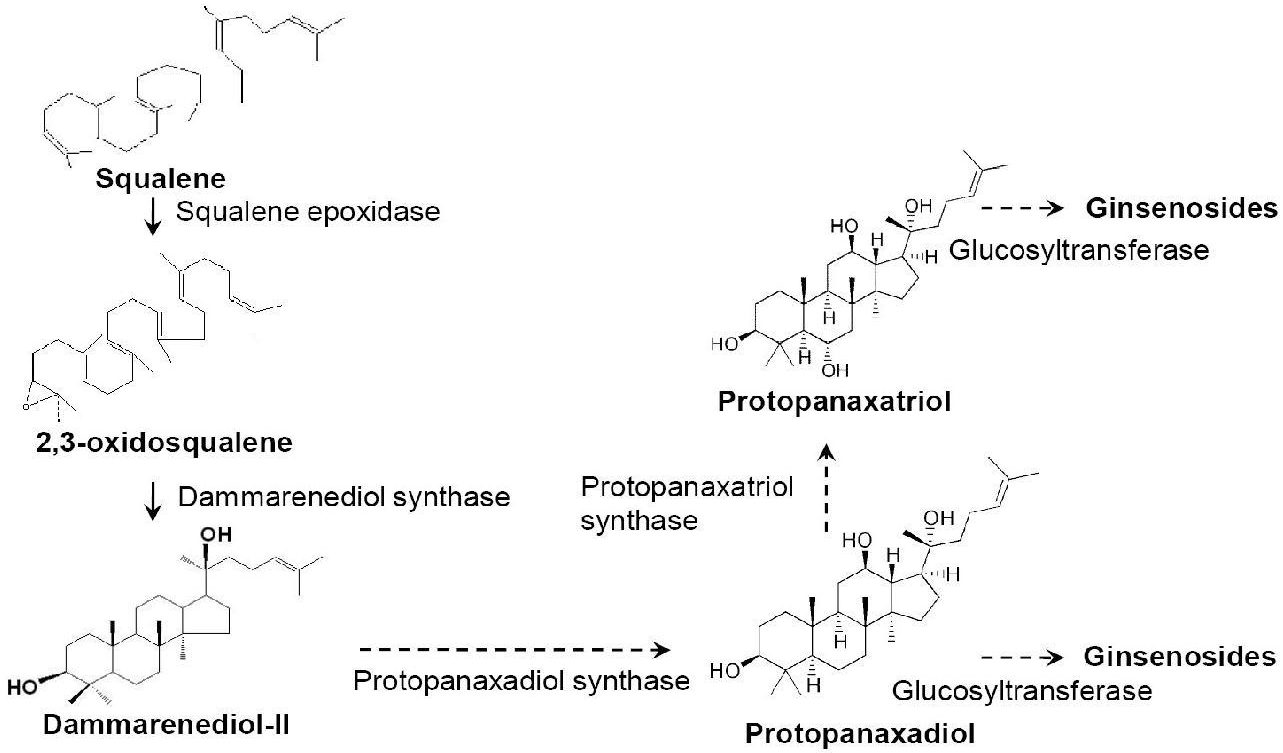

Figure 1. Biosynthetic pathway of ginsenosides from squalene in *P. ginseng*. Triterpene undergoes oxidation, glycosylation and finally converted into triterpene saponins (ginsenosides).

sterol but also for triterpene biosynthesis.

PgSS1 derived from *P. ginseng* was introduced into Siberian ginseng (*Eleutherococcus senticosus*) together with hygromycin phosphotransferase (*HPT*) and green fluorescent protein (*GFP*) through *Agrobacterium*-mediated transformation (Seo et al., 2005). Transgenic *E. senticosus* plants revealed that phytosterols (β-sitosterol and stigmasterol) as well as triterpene saponins (ciwujianosides B, C_1, C_2, C_3, C_4, D_1 and D_2 levels in transgenic *E. senticosus*) were increased by 2 to 2.5 fold. These results suggested that overexpression of *PgSS1* gene by genetic transformation is useful to enhance production of phytosterols and triterpenoids in plants.

Squalene epoxidase

Squalene epoxidase catalyzes the first oxygenation step in phytosterol and triterpenoid saponin biosynthesis and is suggested to represent one of the rate-limiting enzymes in this pathway. Han et al. (2010) investigated the roles of two squalene epoxidase genes (*PgSQE1* and *PgSQE2*) in triterpene and phytosterol biosynthesis in *P. ginseng*. Amino acid sequences deduced from *PgSQE1* and *PgSQE2* share 83% homology, but the N-terminal regions (first 60 amino acids) were highly different. *PgSQE1* mRNA abundantly accumulated in all organs. In contrast, *PgSQE2* was weakly expressed and preferentially in petioles and flower buds. MeJA treatment enhanced the accumulation of *PgSQE1* mRNA in roots, but rather suppressed expression of *PgSQE2*. *In situ* hybridization analysis revealed that both *PgSQE1* and *PgSQE2* mRNAs were accumulated preferentially in vascular bundle tissue and resin ducts of petioles. In *Euphorbia tirucalli*, an oil plant, *in situ* hybridization revealed that *EtSQE* mRNA accumulated prominently in parenchyma cells adjacent to primary laticifers that were located in the inner region of the cortex (Uchida et al., 2007). The phloem is responsible for long-distance transport of assimilates to non-photosynthesizing organs of the plant (Madey et al., 2002) . Phloem and resin duct-specific accumulation of *PgSE1* and *PgSE2* mRNA in *P. ginseng* indicated that these tissue structures represent the metabolically active sites for biosynthesis and transportation of sterol and saponin.

RNA interference (RNAi) of *PgSQE1* in transgenic *P. ginseng* completely suppressed *PgSQE1* transcription. Concomitantly, the interference of *PgSQE1* resulted in reduction of ginsenoside production. Interestingly, silencing of *PgSQE1* in RNAi roots strongly upregulated *PgSQE2* and *PNX* (cycloartenol synthase) and resulted in enhanced phytosterol accumulation. These results indicated that expression of *PgSQE1* and *PgSQE2* were regulated in different manner and *PgSQE2* exhibits some compensational activity when *PgSQE1* gene is under silencing conditions induced by RNAi. *PgSQE1* will regu-

late ginsenoside biosynthesis, but not that of phytosterols in *P. ginseng*. Therefore, overexpression of *PgSQE1* will be useful for the enhanced production of ginsenosides in *P. ginseng* by genetic transformation.

Dammarenediol synthase as a unique group of oxidosqualene cyclase

The first committed step in ginsenoside synthesis is the cyclization of 2,3-oxidosqualene to dammarenediol II by the oxidosqualene cyclase (dammarenediol synthase). The gene encoding dammarenediol synthase was characterized by Han et al. (2006) and Tansakul et al. (2006). Tansakul et al. (2006) reported the *PNA* cDNA (Accession No. AB265170 in DDBJ sequence-database) as dammarenediol synthase genes. Another *DDS* cDNA (accession no. AB122080 in DDBJ/EMBL/GenBank sequence-database) was characterized by Han et al. (2006) . Alignment of the deduced amino acid sequences of two dammarenediol synthase genes (*DDS* and *PNA*) showed that four amino acids from the total were different (Figure 2). Characterization of the two genes was done by functional analysis of heterologous expression in *erg7* yeast mutant (Han et al., 2006; Tansakul et al., 2006). Ectopic expression of *DDS* in yeast mutant (*erg7*) lacking lanosterol synthase resulted in the production of dammarenediol and hydroxydammarenone which were confirmed by LC/APCIMS (Han et al., 2006) but ectopic expression of *PNA* in yeast mutant (*erg7*) resulted in the production of dammarenediol only (Tansakul et al., 2006). This might be due to the differences of amino acid sequences. The deduced amino acids of *DDS* were found to be 56.2, 55.7, 50.5 and 47.9% identical to those of *PNY2* (- amyrin synthase in *P. ginseng*), *PNY* (-amyrin synthase in *P. ginseng*), *PNX* (cycloartenol synthase in *P. ginseng*) and *PNZ* (lanosterol synthase in *P. ginseng*) (Suzuki et al., 2006). The relative low identities of the DDS protein with other OSC proteins of *P. ginseng* suggested that this gene belongs to a new type of OSC gene family in *P. ginseng* as shown in Figure 3. RNAi of *DDS* in transgenic *P. ginseng* resulted in silencing of *DDS* expression which leads to the reduction of ginsenosides production to 84.5% in roots (Han et al., 2006). These results indicated that the expression of *DDS* played a vital role in the biosynthesis of ginsenosides in *P. ginseng.*

PROTOPANAXATRIOL AND GLYCOSYLTRANSFERASES INVOLVED IN GINSENOSIDE HETEROGENEITY

Roots of *P. ginseng* contain at least more than 4% ginsenosides of dry weight (Shibata, 2001). Seven dammarene-type tetracyclic triterpene (ginsenoside Rb1, Rb2, Rc, Rd, Re, Rf and Rg1) are reported as major constituents and only ginsenoside Ro is oleanane- type pentacyclic triterpene, which is a minor component in *P. ginseng*. Each ginsenoside has been shown to have different pharmacological effects, including immune system modulation, antistress, antihyperglycemic, anti-inflammatory, antioxidant, and anticancer effects (Briskin, 2000; Shibata, 2001). The occurrence of dammarene-type triterpene as major compound was restricted to a few species, *P. ginseng* (Kushiro et al., 1997) and *Gynostemma pentaphyllum* (Cui et al., 1999).

Ginsenosides are supposed to be synthesized from dammarenediol II after hydroxylation by cytochrome P450 (Shibuya et al., 2006) and subsequently by the glycosy-lation with glycosyltransferase (Kushiro et al., 1997; Kushiro et al., 1998; Haralampidis et al., 2001; Choi et al., 2005). Both cytochrome P450s and glycosyltransferases exist as supergene families in the plant genome. These ginsenosides are divided into two groups by the structure of aglycone: panaxadiol group (Rb1, Rb2, Rc and Rd) and the panaxatriol group (Rg1, Re, Rf and Rg2). Cytochrome P450 member in *P. ginseng* might be involved in the hydroxylation of the C-12 position of dammarenediol for protopanaxadiol synthesis and the C-6 position of protopanaxadiol for protopanaxatriol synthe-sis, and these two compounds are used as backbones for ginsenosides. Thereafter, biosynthesis of ginsenosides from triterpene aglycone involves glycosylation at the C-3 and C-20 hydroxyl positions on the skeleton for proto-panaxadiol type and the C-6 and C-20 positions for protopanaxatriol type ginsenoside (Figure 1). A hydroxy-lase enzyme catalyzing the biosynthesis of protopana-xatriol from protopanaxadiol (P6H) was identified in *P. notoginseng*. Activity of P6H was dependent on NADPH and molecular oxygen. The enzymatic reaction was inhibited by carbon monoxide and partially reversible upon illumination with blue light, and sensitive to cytochrome P450 inhibitors (Yue et al., 2008). In *P. notoginseng*, a ginsenoside glucosyltransferase, UDPG: ginsenoside Rd glucosyltransferase, was identified (Yue and Zhong, 2005). Ginseoside Rd glucosyltransferase catalyzes the formation of ginsenoside Rb1 from ginsenoside Rd. This enzyme had a molecular mass of 36 kDa and is strongly affected by many metal cations and had a high potency of biotransforming ginsenoside Rd to Rb1. The information of two enzyme works in *P. notoginseng* will help for identification and characteri-zation of protopanaxatriol and glycosyltransferases involved in regulating ginsenoside heterogeneity in *P. ginseng*. However, the genes determining the protopanaxadiol and protopanaxatriol synthase and glucosyltransferase are still uncharacterized.

Concluding Remarks

The recent progresses of ginseng biotechnology for gene mining and functional analysis of genes involved in ginsenoside biosynthesis will provide the information necessary to design new ginseng by molecular breeding. The saponin metabolic engineering might be a promising

Figure 2. Alignment of the deduced amino acid sequences of two types of dammarenediol synthase in *P. ginseng*. The DDBJ/GenBank/EMBL accession numbers of the sequences are AB122080 (*DDS*), and AB265170 (*PNA*).

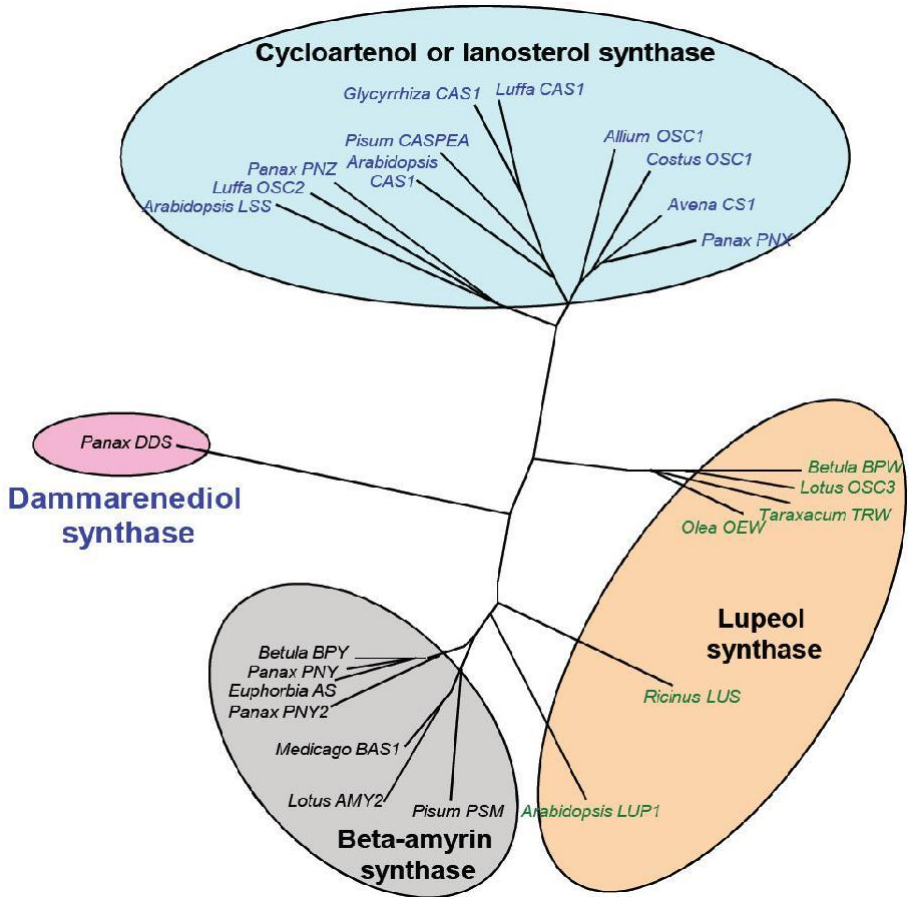

Figure 3. Phylogenic tree of the deduced amino acid sequences of *DDS* and other plant OSCs. The DDBJ/GenBank/EMBL accession numbers of the sequences are AB122080 for dammarenediol synthase (*DDS*) in *P. ginseng*, AB055512 for beta- amyrin synthase (*BPY*) in *Betula platyphylla*, AB009030 for beta-amyrin synthase (*PNY*) in *P. ginseng*, AB206469 for beta-amyrin synthase (*AS*) in *Euphorbia tirucalli*, AB014057 for beta-amyrin synthase (*PNY2*) in *P. ginseng*, AJ430607 for beta-amyrin synthase (*BAS1*) in *Medicago truncatula*, AF478455 for multifunctional beta-amyrin synthase (*AMY2*) in *Lotus japonicus*, U49919 for lupeol synthase (*LUP1*) in *Arabidopsis thaliana*, DQ268869 for lupeol synthase (*LUS*) in *Ricinus communis*, AB025343 for lupeol synthase (*OEW*) in *Olea europaea*, AB025345 for lupeol synthase (*TRW*) in *Taraxacum officinale*, AB181245 for lupeol synthase (*OSC3*) in *Lotus japonicus*, AB055511 for lupeol synthase (*BPW*) in *Betula platyphylla*, AB009029 for cycloartenol synthase (*PNX*) in *P. ginseng*, AJ311790 for cycloartenol synthase (*CS1*) in *Avena strigosa*, AB058507 for cycloartenol synthase (*OSC1*) in *Costus speciosus*, AB033334 for cycloartenol synthase (*CAS1*) in *Luffa aegyptiaca*, AB025968 for cycloartenol synthase (*CAS1*) in *Glycyrrhiza glabra*, D89619 for cycloartenol synthase (*CASPEA*) in *Pisum sativum*, A49398 for cycloartenol synthase (*CAS1*) in *Arabidopsis thaliana*, AB009031 for lanosterol synthase (*PNZ*) in *P. ginseng*, DQ508794 for lanosterol synthase (*LSS*) in *Arabidopsis thaliana*. Phylogenetic tree of plant OSCs distances between each clone and group are calculated with the program CLUSTAL W. The distances between each clone were calculated using CLUSTAL W.

area of ginseng biotechnology.

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REFERENCES

Abe I, Rohmer M, Prestwich GD (1993) Enzymatic cyclization of squalene and oxidosqualene to sterols and triterpenes. Chem. Rev. 93: 2189-2206.

Asaka I, Li I, Hirotani M, Asada Y, Furuya T (1993) Production of ginsenoside saponins by culturing ginseng (*Panax ginseng*) embryogenic tissues in bioreactors. Biotechnol. Lett. 15:1259-1264

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. Biotechnol Lett. 28:1163-1166

Bang KH, Kim OT, Jung SJ, Kim YC, Park HW, Cha SW (2009) Molecular characterization of ginseng farnesyl diphosphate synthase gene and its up-regulation by methyl jasmonate. Biol. Plant (in press)

Briskin DP (2000) Medicinal plants and phytomedicines. Linking plant biochemistry and physiology to human health. Plant Physiol. 124:507-514

Choi DW, Jung JD, Ha YI, Park HW, In DS, Chung HJ, Liu JR (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. Rep. 23:557-566.

Choi SM, Son SH, Kwon OW, Seon JH, Paek KY (2000) Pilot-scale culture of adventitious roots of ginseng in a bioreactor system. Plant Cell Tiss. Organ Cult. 62:187-193

Coleman CI, Hebert JH, Reddy P (2003) The effects of *Panax ginseng* on quality of life. J. Clin. Pharm. Ther. 28:5-15

Cui JF, Eneroth P, Bruhn JC (1999) *Gynostemma pentaphyllum*: identification of major sapogenins and differentiation from *Panax* species. Eur. J. Pharm. Sci. 8:187-191

Dey L, Xie JT, Wang A, Wu J, Maleckar SA, Yuan CS (2003) Anti-hyperglycemic effects of ginseng: comparison between root and berry. Phytomedicine 10:600-605

Ellis JM, Reddy P (2002) Effects of *Panax ginseng* on quality of life. Ann. Pharmacther. 36:375-379

Furuya T, Ushiyama K (1994) Ginseng production in cultures of *Panax ginseng* cells. In: Shargool P, Ngo TT (eds) Biotechnological application of plant cultures. CRC, Boca Raton, pp 1-22

Han JY, Kwon YS, Yang DC, Jung YR, Choi YE (2006) Expression and RNA interference-induced silencing of the dammarenediol synthase gene in *Panax ginseng*. Plant Cell Physiol. 47:1653-1662

Han JY, In JG, Kwon YS, Choi YE (2010) Regulation of ginsenoside and phytosterol biosynthesis by RNA interferences of squalene epoxidase gene in *Panax ginseng*. Phytochemistry. 71:36-46.

Haralampidis K, Trojanowska M, Oshourn AE (2001) Biosynthesis of triterpenoid saponin in plants. Adv Biochem Eng Biotechnol 75:31-49

Harrison MD (1990) The biosynthesis of triterpenoids, steroids, and carotenoids. Nat. Prod. Rep. 7:459-484

Hu FX, Zhong JJ (2007) Role of jasmonic acid in alteration of ginsenoside heterogeneity in elicited cell cultures of *Panax notoginseng*. J. Biosci. Bioeng. 104:513-516

Jeong GT, Park DH, Hwang B, Woo JC (2003) Comparison of growth characteristics of *Panax ginseng* hairy roots in various bioreactors. Appl. Biochem. Biotechnol. 107:493-503

Jung JD, Park HW, Hahn Y, Hur CG, In DS, Chung HJ, Liu JR, Choi DW (2003) Discovery of genes for ginsenoside biosynthesis by analysis of ginseng expressed sequence tag. Plant Cell Rep. 22:224-230

Kevers C, Jacques P, Thonart P, Gaspar T (1999) In vitro root cultures of *Panax ginseng* and *Panax quinquefolium*. Plant Growth Regul. 27:173-178

Kiefer D, Pantuso T (2003) *Panax ginseng*. Am. Fam. Physician 68: 1539-1542.

Kim OT, Bang KH, Kim YC, Hyun DY, Kim MY, Cha SW (2009) Upregulation of ginsenoside and gene expression related to triterpene biosynthesis in ginseng hairy root cultures elicited by methyl jasmonate. Plant Cell Tiss. Organ Cult. 98:25-33

Kim YS, Hahn EJ, Murthy HN, Paek KY (2004) Adventitious root growth and ginsenoside accumulation in *Panax ginseng* cultures as affected by methyl jasmonate. Biotechnol. Lett. 26:1619-1622

Kim YS, Yeung EC, Hahn EJ, Paek KY (2007) Combined effects of phytohormone, indole-3-butyric acid, and methyl jasmonate on root growth and ginsenoside production in adventitious root cultures of *Panax ginseng* C.A. Meyer. Biotechnol. Lett. 29:1789-1792

Kushiro T, Ohno Y, Shibuya Y, Ebizuka Y (1997) In vitro conversion of 2,3-oxidosqualene into dammarenediol by *Panax ginseng* microsomes. Biol. Pharm. Bull. 20: 292-294.

Kushiro T, Shibuya Y, Ebizuka Y (1998) -amyrin synthase: cloning of oxidosqualene cyclase that catalyze the formation of the most popular triterpene among higher plants. Eur. J. Biochem. 256:238-244.

Kuzuyama T (2002) Mevalonate and nonmevalonate pathways for the biosynthesis of isoprene units. Biosci. Biotechnol. Biochem. 66:1619-1627

Lee MH, Jeong JH, Seo JW, Shin CG, Kim YS, In JG, Yang DC, Yi JS, Choi YE (2004) Enhanced triterpene and phyotsterol biosynthesis in *Panax ginseng* overexpressing squalene synthase gene. Plant Cell Physiol. 45:976-984

Madey E, Nowack LM, Thompson JE (2002) Isolation and characterization of lipid in phloem sap of canola. Planta 214:625-634

Shibata S (2001) Chemistry and cancer preventing activities of ginseng saponins and some related triterpenoid compounds. J. Kor. Med. Sci. 16 [Suppl]:S28-S37

Shibuya M, Hoshino M, Katsube Y, Hayashi H, Kushiro T, Ebizuka Y (2006) Identification of -amyrin and sophoradiol 24-hydroxylase by expressed sequence tag mining and functional expression assay. FEBS J. 273:948-959

Seo JW, Jeong JH, Shin CG, Lo SC, Han SS, Yu KW, Harada E, Han JY, Choi YE (2005) Overexpression of squalene synthase in *Eleutherococcus senticosus* increases phytosterol and triterpene accumulation. Phytochemistry 66:869-877

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

Sticher O (1998) Getting to the root of ginseng. Chemtech 28:26-32. Suzuki M, Xiang T, Ohyama K, Seki H, Saito K, Muranaka T, Hayashi H, Katsube Y, Kushiro T, Shibuya M, Ebizuka Y (2006) Lanosterol synthase in dicotyledonous plants. Plant Cell Physiol 47:565-571.

Tansakul P, Shibuya M, Kushiro T, Ebizuka Y (2006). Dammarenediol - II synthase, the first dedicated enzyme for ginsenoside biosynthesis, in *Panax ginseng*. FEBS Lett. 580:5143-5149.

Uchida H, Sugiyama R, Nakayachi O, Takemura M, Ohyama K (2007) Expression of the gene for sterol-biosynthesis enzyme squalene epoxidase in parenchyma cells of the oil plant, *Euphorbia tirucalli*. Planta 226:1109-1115.

Vogler BK, Pittler MH, Ernst E (1999) The efficacy of ginseng. A systematic review of randomized clinical trials. Eur. J. Clin. Pharmacol. 55:567-575.

Wang W, Zhong JJ (2002) Manipulation of ginsenoside heterogeneity in cell cultures of Panax notoginseng by addition of jasmonates. J. Biosci. Bioeng. 93:48-53.

Wu JY, Zhong JJ (1999). Production of ginseng and its bioactive components in plant cell culture: current technological and applied aspects. J. Biotechnol. 68:89-99.

Yoshikawa T, Furuya T (1987). Saponin production by cultures of *Panax ginseng* transformed with *Agrobacterium rhizogens*. Plant Cell Rep. 6:449-453.

Yu KW, Gao WY, Son SH, Paek KY (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 Cell Dev. Biol. –Plant 36:424-428.

Yu KW, Gao WY, Hahn EJ, Paek KY (2002). Jasmonic acid improves ginsenoside accumulation in adventitious root culture of *Panax ginseng* C.A. Meyer. Biochem. Eng. J. 3596:1-5.

Yue CJ, Zhong JJ (2005). Purification and characterization of UDPG:ginsenoside Rd glucosyltransferase from suspended cells of Panax notoginseng. Process Biochem. 40: 3742-3748.

Yue CJ, Zhou X, Zhong JJ (2008) Protopanaxadiol 6-hydroxylase and its role in regulating the ginsenoside heterogeneity in *Panax notoginseng* cells. Biotechnol. Bioeng. 100: 933-940.

Yun TK (2001) *Panax ginseng*-a non-organ-specific cancer preventive? Lancet Oncol. 2: 49-55.