Plant Omics Journal POJ

POJ 5(1):28-32 (2012) ISSN:1836-3644

Overexpressing Arabidopsis jasmonic acid carboxyl methyltransferase (*AtJMT*) results in stimulation of root growth and ginsenoside heterogeneity in *Panax ginseng*

Yun-Soo Kim, Jung-Yeon Han, Soon Lim, Hyun-Jung Kim, Mi-Hyun Lee, Yong-Eui Choi*

Division of Forest Resources, College of Forest Sciences, Kangwon National University, Chuncheon 200-701, South Korea

***Corresponding author: yechoi@kangwon.ac.kr**

Abstract

Methyl jasmonate (MeJA) triggers the production of secondary metabolites in plants and participates in a diverse range of plant developmental processes. MeJA is derived from jasmonic acid (JA) via the octadecanoid pathway and the reaction is catalyzed by jasmonic acid carboxyl methyltransferase (JMT). In this study, transgenic *Panax ginseng* roots were constructed to express an Arabidopsis jasmonic acid carboxyl methyltransferase (*AtJMT*). The transgenic lines exhibited high expression of genes (*PgSS1*, *PgSE1*, and *PgDDS*) involved in ginsenoside biosynthetic pathways as well as a MeJA-responsive gene (*PgPR10-2*). These alterations of the gene expressions led to 3-fold increase in the growth of transgenic roots. Overexpression of *AtJMT* gene strongly affected the ginsenoside heterogeneity (protopanaxadiol/protopanaxatriol ratio) although total ginsenoside accumulation was slightly increased in transgenic roots. Protopanaxadiol group of ginsenosides (Rb1, Rc, and Rb2) increased about 2-fold in transgenic roots compared to those in wild-type. The results suggest that overexpression of *AtJMT* stimulate not only the growth of roots but also the production of protopanaxadiol-group in transgenic roots.

Keywords : Adventitious root cultures; Methyl jasmonate; Pathogen-related genes; Protopanaxadiol; Protopanaxatriol.
Abbreviations : *AtJMT* - Arabidopsis jasmonic acid carboxyl methyltransferase; HPLC - High performance liquid chromatography; IBA - Indole-3-butyric acid; JA - Jasmonic acid; JMT - Jasmonic acid carboxyl methyltransferase; MeJA - Methyl jasmonate; MS medium - Murashige and Skoog medium; PgSS - *Panax ginseng* squalene synthase; PgSE - *Panax ginseng* squalene epoxidase; PgDDS - *Panax ginseng* dammarenediol synthase.

Introduction

Ginsenosides are triterpene saponins and found exclusively in the plant genus *Panax*, which have numerous pharmacological effects including immune system modulation, anti-stress activities, anti-hyperglycemic activities, anti-inflammatory, anti-oxidant, and anti-cancer properties (Vogler et al., 1999; Shibata, 2001; Yun, 2001). Ginsenosides are synthesized through the isoprenoid pathway in which squalene acts a committed precursor. The squalene is synthesized by squalene synthase from farneyldiphosphate (Kuzuyama, 2002; Lee et al., 2004). Subsequent reactions with squalene epoxidase yields 2,3-oxidosqualene. The cyclization of 2,3-oxidosqualene into dammarenediol is catalyzed by dammarenediol-II synthase (Han et al., 2006; Tansakul et al., 2006). Dammarenediol is used as a precursor to yield dammarene-type triterpenes which may be converted to each ginsenoside by cytochrome P450-dependent mono-oxygenases and glucosyltransferases (see Fig. 1; Haralampidis et al., 2001; Kim et al., 2009a). To date, there have been many approaches to increase the yield of ginsenosides from ginseng cell or organ cultures (Wu and Zhong, 1999; Paek et al., 2009). The treatment of methyl jasmonate (MeJA) is the most efficient method by which ginsenosides were highly accumulated in cultured materials of ginseng (Kim et al., 2004; Bae et al., 2006; Ali et al., 2006; Kim et al., 2007). Recently, molecular studies of genes involved in ginsenoside biosynthetic pathways have revealed that exogenously applied MeJA stimulated the accumulation of gene transcripts involved in ginsenoside biosynthesis, and thereby the ginsenoside production were enhanced in ginseng adventitious roots (Lee et al., 2004; Han et al., 2006; Kim et al., 2009b; Han et al., 2010). MeJA are distributed throughout higher plants in which it is derived from jasmonic acid (JA) via the octadecanoid pathway and the reaction is catalyzed by jasmonic acid carboxyl methyltransferase (JMT) (Creelman and Mullet 1997). A JMT gene (*AtJMT*) has been isolated in Arabidopsis (*Arabidopsis thaliana*), the transgenic Arabidopsis overexpressing *AtJMT* exhibited elevated levels of endogenous MeJA, and the transgenic plants also enhanced the expression of JA-response genes and defense-related genes (Seo et al., 2001). In recent report, the *AtJMT* gene was introduced into various crops such as rice and potato and the overexpression of *AtJMT* increased the levels of endogenous MeJA in their transgenic lines (Kim et al., 2009c; Sohn et al., 2011). The increased MeJA in transgenic lines affected on agricultural traits such as their yields and organ development. Both examples in the endogenous MeJA and the actions of exogenously applied MeJA point that the enhanced endogenous MeJA in transgenic plants overexpressing *AtJMT* could profoundly affect morphological alterations (Kim et al., 2009c; Sohn et al., 2011) as well as secondary metabolites (Yukimune et al., 1996; Kim et al., 2004; Ali et al., 2006). We thus proposed that transgenic ginseng adventitious roots overexpressing *AtJMT* would be expected to increase the ginsenoside biosynthesis. In this study, transgenic ginseng adventitious roots overexpressing the *AtJMT* by

Agrobacterium-mediated transformation were generated. Changes of morphology and ginsenoside accumulation were investigated in the transgenic roots. The results showed that overexpression of *AtJMT* stimulated the root growth and the protopanaxadiol-type of ginsenosides in transgenic adventitious roots of *P. ginseng*.

Results and discussion

Generation of transgenic adventitious roots

To analyze functional role of *AtJMT* in transgenic ginseng adventitious roots, T-DNA (Fig. 2a) of the binary vector containing the *AtJMT* gene and a selection marker gene was introduced into ginseng adventitious roots via *Agrobacterium*-mediated transformation (Han and Choi, 2009). Kanamycin-resistant roots induced and proliferated on half strength MS medium supplemented with 3 mg l^{-1} IBA, 2% sucrose, and 100 mg l^{-1} kanamycin. After proliferation on the selection medium, 4 lines of kanamycin-resistant roots were confirmed by detection of *AtJMT* mRNA accumulation by RT-PCR (Fig. 2b). The results showed the introduced *AtJMT* gene was actively transcribed in transgenic adventitious roots.

Expression of ginsenoside biosynthetic genes in transgenic adventitious roots

The overexpression of *AtJMT* in transgenic lines increased the transcript accumulation of ginsenoside biosynthetic genes including *PgSS1*, *PgSE1*, and *PgDDS* (Fig. 2c). The transcript accumulations of *PgSS1, PgSE1,* and *PgDDS* in transgenic lines were higher than that in wild-type roots. *PgDDS* is a crucial gene encoding dammarenediol synthase by which tetracyclic triterpene ginsenoside skeletons is constructed in ginseng plants (see Fig. 1; Han et al., 2006). These results suggested that the overexpression of *AtJMT* in transgenic ginseng adventitious roots led to the induction of genes involved in ginsenoside biosynthesis. The enhanced ginsenoside biosynthetic genes (*PgSS1, PgSE1,* and *PgDDS*) in transgenic lines are similar to exogenously applied MeJA effects on gene regulation in ginseng adventitious roots (Lee et al., 2004; Han et al., 2006).

Expression of a JA-responsive gene in transgenic adventitious roots

Previously, it was revealed that the overexpression of *AtJMT* in transgenic plants led to stimulate the JA-responsive genes (Seo et al., 2001; Sohn et al., 2011). The stimulated accumulations of JA-responsive genes have been considered to be induced by the increased MeJA levels in the *AtJMT*-overexpressing transgenic plants. Herein, we investigated the induction of *PgPR10-2*, a JA-responsive gene in *Panax ginseng* (Pulla et al., 2010), in wild-type ginseng adventitious roots subjected to different concentration of MeJA (Fig. 3a). The results showed that MeJA stimulated the expression of *PgPR10-2* with its increasing dose. This finding suggested that *PgPR10-2* is actively inducible in MeJA treatment as well as JA and the elevated transcripts are caused by endogenously-produced MeJA (Sohn et al., 2011).
Based on the MeJA-dependent enhancement of *PgPR10-2* accumulation, accumulation of *PgPR10-2* mRNA was analyzed in transgenic adventitious roots. As shown to Fig. 3b, the transcripts of *PgPR10-2* were highly accumulated in all transgenic roots compared to wild type root. Collectively, these observations suggested that the increased transcript accumulations of *PgPR10-2* mRNA could be resulted from endogenous MeJA accumulation in transgenic ginseng adventitious roots.

Morphology and growth of transgenic roots

To evaluate ginsenoside production in transgenic roots overexpressing *AtJMT*, the root growth and the ginsenoside content were examined in two selected line S13 and S18, which showed the highest levels in *PgDDS* expression (see Fig. 2c). Morphological analysis of transgenic and wild type roots after 40 days of cultures revealed that lateral roots in transgenic lines had thicker than those of wild-type roots (Fig. 4). This morphological change in transgenic roots led to the increase of root dry weight (Fig. 4d). The dry weights in two transgenic lines were approximately 3-fold increases compared to those of wild-type. This result is corresponded with the previous report in which overexpression of jasmonic acid carboxyl methyltransferase increases tuber yield and size in transgenic potato (Sohn et al., 2011). These results are inconsistent with our previous reports in which exogeneously applied MeJA decreased the growth of adventitious roots (Kim et al., 2004; Bae et al., 2006; Kim et al., 2007). However, they treated a high concentration ($\geq$ 100 μM) of MeJA that inhibited the growth of ginseng adventitious roots by preventing cell division (Kim et al., 2007).

Alteration of ginsenoside heterogeneity in transgenic lines

Ginsenoside contents in the tested materials were determined by HPLC. The analysis revealed that only slight increase of total ginsenoside contents in transgenic roots (Fig. 5a). However, there are clear differences in the ratio of protopanaxadiol (Rb1, Rc, Rb2, and Rd) and protopanaxatriol (Re, Rg1, and Rf) groups of ginsenosides. The contents of protopanaxadiol group of ginsenosides increased about 2-fold in two transgenic root lines compared to those in wild-type, whereas the contents of protopanaxatriol group of ginsenosides decreased 1.8-fold in the transgenic lines (Fig. 5b). The HPLC chromatograms of wild-type and transgenic line (S18) were presented in Fig. 6. These results suggested that overexpressions of *AtJMT* stimulated the MeJA-responsive *PgPR10-2* gene and the ginsenoside biosynthesis-related genes which in turn affected to the root growth and ginsenoside accumulation. Particularly, the significant change on ginsenoside heterogeneity in transgenic root lines is consistent with our previous works in which protopanaxadiol-type (Rb group) saponin contents had increased by 11-fold with MeJA elicitation compared to control (0 μM MeJA), whereas protopanaxatriol-type (Rg group) saponin contents had slightly decreased with MeJA elicitation (Kim et al., 2004). Finally, the potential amounts of ginsenoside production were calculated by multiplying root dry weight and ginsenoside content (Fig. 5c). The results showed that the ginsenoside productions in transgenic line S13 and S18 were 2.51 and 2.71 mg $dish^{-1}$, indicating 3.3- and 3.6-fold higher than that in wild-type roots, respectively. Also, the protopanaxadiol production was calculated together with protopanaxatriol in transgenic roots. The results showed that the protopanaxadiol production was increased to about 1.5-fold in transgenic root lines compared to protopanaxatriol production (Fig. 5d). In conclusion, overexpression of the *AtJMT* gene increased the expression of *PgPR10-2* gene (a MeJA-responsive gene) in transgenic root lines. The enhanced expression of *AtJMT* gene in transgenic lines stimulated the transcript accumulation of ginsenoside-biosynthetic genes. These changes in gene regulation finally led to increases in the root growth and the ginsenoside

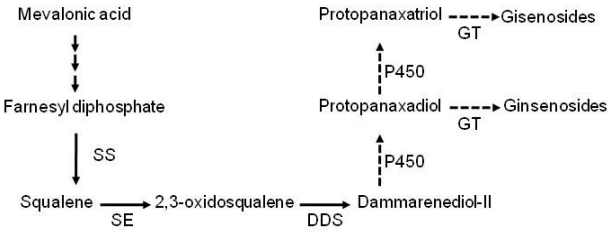

Fig 1. Putative ginsenoside biosynthetic pathways in *Panax ginseng*. SS, squalene synthase; SE, squalene epoxidase; DDS, dammarenediol synthase; P450, cytochrome P450; GT, glucosyltransferase. The dotted lines indicate the as yet uncharacterized regions of the pathway.

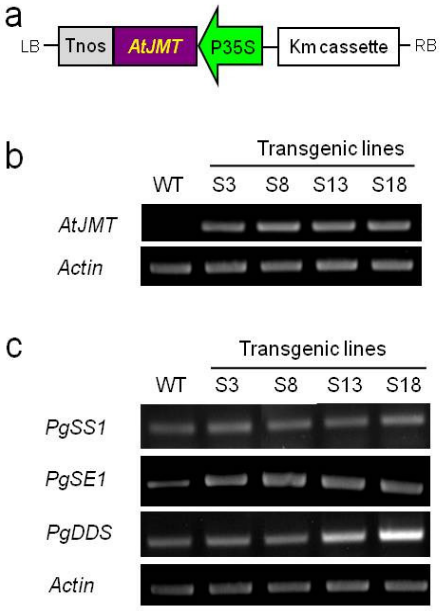

Fig 2. Generation of transgenic ginseng adventitious roots overexpressing *AtJMT* gne. (**a**) Schematic illustration of the T-DNA region of pCaJMT binary vector used in adventitious root transformation. LB, left boader; RB, right boader; P35S, CaMV 35S promoter; Tnos, nopaline synthase terminator. (**b**) *AtJMT* transcript accumulations by RT-PCR analysis in wild-type and transgenic root lines. (**c**) Transcript accumulations of genes involved in ginsenoside biosynthesis (*PgSS1*, *PgSE1*, and *PgDDS*) in wild type and transgenic roots. The actin gene was used as the standard for RNA loading.

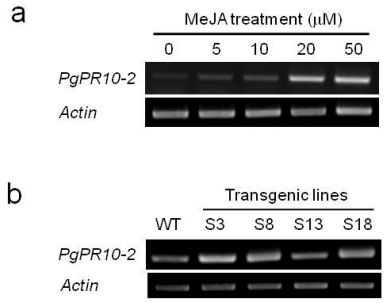

Fig 3. Expression of *PgPR10-2* by RT-PCR. (**a**) Transcript accumulations of *PgPR10-2* after 24 h of 5, 10, 20, and 50 μM MeJA treatment. (**b**) Transcripts of *PgPR10-2* in wild type and transgenic roots. The actin gene was used as the standard for RNA loading.

heterogeneity in transgenic lines compared to wild-type.

Materials and methods

Plant materials and genetic transformation

Adventitious roots of *Panax ginseng* were induced from leaf explants of *in vitro* ginseng plants and maintained by sub-culturing on a half strength MS (Murahsige and Skoog, 1962) medium containing 3 mg l^{-1} IBA and 2% sucrose at 3 week intervals (Lee et al., 2004). Genetic transformation of ginseng adventitious roots was carried out as described in our previous reports (Han and Choi, 2009). The binary vector containing Arabidopsis (*A. thaliana*) *JMT* (*AtJMT*) was kindly provided from Prof. Yang-Do Choi in Seoul University (Fig. 2a: Seo et al., 2001). The binary vector was introduced into *A. tumefaciens* C58C1 for transformation. Briefly, ginseng adventitious roots sectioned to 10 mm length were infected by *A. tumefaciens*. The infected materials were placed on a half strength MS medium supplemented with 3 mg l^{-1} IBA for 3 days and transferred onto fresh medium with 3 mg l^{-1} IBA and 300 mg l^{-1} cefotaxime to remove the bacteria for two weeks. Thereafter, root explants were subcultured at 3 week intervals onto medium with 3 mg l^{-1} IBA, 100 mg l^{-1} kanamycin and 300 mg l^{-1} cefotaxime for selection of kanamycin resistant roots. The root segments selected on media containing kanamycin were confirmed for transgene expression by RT-PCR (see below). Resulting transformants were considered as lines and proliferated in growth chamber at 24°C under continuous dark for further experiments.

RT-PCR analysis

Total RNA was prepared from ginseng adventitious roots using an RNeasy Plant Mini Kit (Qiagen) and subjected to RT-PCR. First-strand cDNA was prepared using oligo(dT) as primer with ImProm-II Reverse Transcription System (Promega, USA).

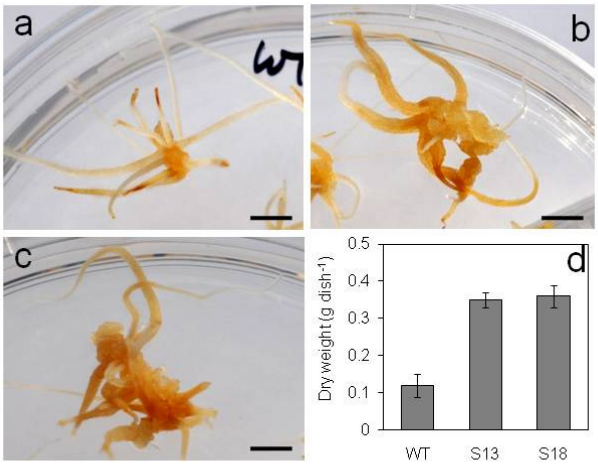

Fig 4. Morphological changes and growth yields in transgenic ginseng adventitious roots overexpressing *AtJMT* gene. Root morphologies in wild type (**a**), transgenic line S13 (**b**), and transgenic line S18 (**c**) after 40 days of cultures. Bars indicate 0.5 cm-length. (**d**) Root dry weight in wild type and transgenic lines at 40 days of cultures. Each dry weight was calculated by 10 explants/petridish.

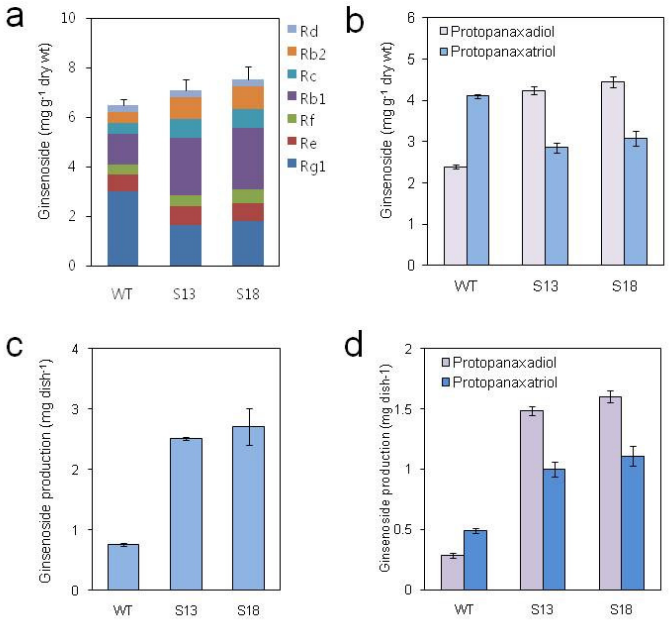

Fig 5. Ginsenoside production in transgenic ginseng adventitious roots. Accumulation of each ginsenoside (**a**) and protopanaxadiol (ginsenoside Rb1+Rb2+Rc+Rd) and protopanaxatiol (ginsenoside Rg1+Re+Rf) (**b**) in wild type and tranasgenic root line S13 and S18 after 40 days of cultures. Ginsenoside productions (**c, d**) were calculated by multiplying ginsenoside content and dry weight in wild type and transgenic lines, respectively.

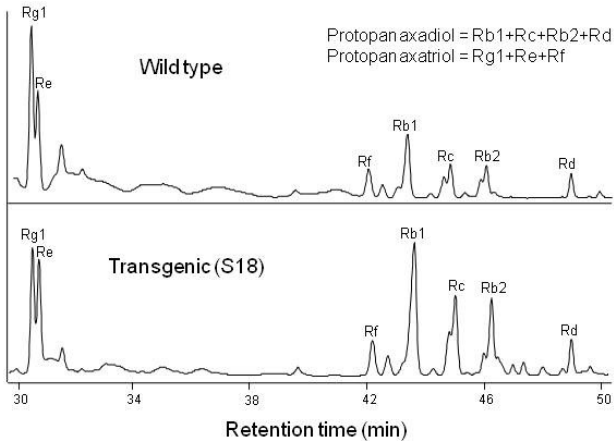

Fig 6. HPLC chromatograms in wild type (**a**) and transgenic line S18 (**b**). The dry masses for analysis were collected from each root harvested after 40 days of culture.

The cDNA was used as template for PCR (denaturation at 96°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s). Primer sets were 5'-CAATTCTGGAAAAGTATGAT -GGG-3'/5'-TCG AACAAGGTGGAGCTTGATAT-3' (for *AtJMT*; accession no. AY008434); 5'-ATGGGTGTCCA-AAAGACCGA-3'/5'-CTAATTTGCTAGGAGGTAAGCTTC-3' (for *PgPR10-2*; GU086324); 5'-ATGGGGAGAGG-AAGGGTA-3'/5'-TTATTGGTTAACATGACGAAGC-3' (for *PgSS1*; AB115496); 5'-ATGAATTCATCTTCTTCTAGTA-CTACT-3'/5'-TTAGTGAATGGGGGGAGCTC-3' (for *PgSE1*; AB122078); 5'-ATGTGGAAGCTGAAGGTTGCTC-3'/5'-TTAAATTTTGAGCTGCTGGTGCT-3' (for *PgDDS*; AB122080); 5'-CGTGATCTTACAGATAGCTTCATGA-3'/5'-AGAGAAGCTAAGATTGATCCTCC-3' (for *Actin*; AY907207). PCR products were analyzed by electrophoresis in 1% agarose gels.

MeJA treatment

MeJA at different concentration (0, 5, 10, 20, and 50 μM) was added into each 50 ml conical tube containing 30 ml liquid half strength MS medium supplemented with 3 mg l^{-1} IBA and 2% sucrose, respectively. Ten adventitious roots cultured for 4 weeks were transferred into each conical tube containing MeJA. The conical tubes were cultured in 120 rpm rotary shaker at 24°C dark condition for 24 h. MeJA was purchased from Duchefa (the Netherlands) and used after dilution with 40% aq. EtOH. In case of control (0 μM MeJA), 40% aq. EtOH was applied to avoid the chemical effect.

Ginsenoside analysis by HPLC

Extraction of ginsenosides was the same as previously described (Han et al., 2006). The HPLC separation was performed on a C18 column (5 μm, 4.6×250 mm, Agilent, Wilmington, USA) with water and acetonitrile as mobile phase. The time and ratios of water and acetonitrile followed the protocol of Samukawa et al. (1995). The flow rate of the mobile phase was 1.0 ml min^{-1}, and ginsenosides were monitored at a wavelength of 202 nm. Each ginsenoside was compared with the authentic ginsenoside samples purchased

from ChromaDex Inc (California, USA). Quantitative analysis was performed with one-point curve method using external standards of authentic ginsenosides.

Acknowledgements
This work was supported by a grant from the World Class University Project of Korea.

References

Ali MB, Yu K-W, Hahn E-J, Paek K-Y (2006) Methyl jasmonate and salicylic acid elicitation induces ginsenosides accumulation, enzymatic and non-enzymatic antioxidant in suspension culture *Panax ginseng* roots in bioreactors. Plant Cell Rep 25:613-620

Bae K-H, Choi Y-E, Shin C-G, Kim Y-Y, Kim Y-S (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

Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. Annu Rev Plant Physiol Plant Mol Biol 48:355-381

Han J-Y, Choi Y-E (2009) Rapid induction of *Agrobacterium tumefaciens*-mediated transgenic roots directly from adventitious roots in *Panax ginseng*. Plant Cell Tiss Organ Cult 96:143–149

Han J-Y, Kwon Y-S, Yang D-C, Jung Y-R, Choi Y-E (2006) Expression and RNA interference-induced silencing of the dammarenediol synthase gene in *Panax ginseng*. Plant Cell Physiol 47:1653-1662

Han J-Y, In J-G, Kwon Y-S, Choi Y-E (2010) Regulation of ginsenoside and phytosterol biosynthesis by RNA interferences of squalene epoxidase gene in *Panax ginseng*. Phytochem 74:36-46

Haralampidis K, Trojanowska M, Osbourn AE (2002) Biosynthesis of triterpenoid saponin in plants. Adv Biochem Eng Biotechnol 75: 31049

Kim E-H, Kim Y-S, Park S-H, Koo Y-J, Choi Y-D, Chung Y-Y, Lee I-J, Kim J-K (2009c) Methyl jasmonate reduces grain yield by mediating stress signals to alter spikelet development in rice. Plant Physiol 149:1751-1760

Kim O-T, Bang K-H, Kim Y-C, Hyun D-Y, Kim M-Y, Cha S-W (2009b) 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 Y-S, Hahn E-J, Murthy HN, Paek K-Y (2004) Adventitious root growth and ginsenoside accumulation in *Panax ginseng* cultures as affected by methyl jasmonate. Biotechnol Lett 26:1619-1622

Kim Y-S, Han J-Y, Lim S, Choi Y-E (2009a) Ginseng metabolic engineering: Regulation of genes related to ginsenoside biosynthesis. J Med Plant Res 3:1270-1276

Kim Y-S, Yeung E-C, Hahn E-J, Paek K-Y (2007) Combined effects of phytohormone, indole-3-butyric acid, and methyl jasmonate on root growth and ginsenoside accumulation in adventitious root cultures of *Panax ginseng* C.A. Meyer. Biotechnol Lett 29:1789-1792

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

Lee M-H, Jeong J-H, Seo J-W, Shin C-G, Kim Y-S, In J-G, Yang D-C, Yi J-S, Choi Y-E (2004) Enhanced triterpene and phytosterol biosynthesis in *Panax ginseng* overexpressing squalene synthase gene. Plant Cell Physiol 45:976-984

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue. Physiol Plant 15:473-497

Paek K-Y, Murthy HN, Hahn E-J, Zhong JJ (2009) Large scale culture of ginseng adventitious roots for production of ginsenosides. Adv Biochem Engin/Biotechnol 113:151-176

Pulla RK, Lee O-R, In J-G, Kim Y-J, Senthil K, Yang D-C (2010) Expression and functional characterization of pathogenesis-related protein family 10 gene, *PgPR10-2*, from *Panax ginseng* C.A. Meyer. Physiol Mol Plant Path 74:323-329

Samukawa K, Yamashita H, Matsuda H, Kubo M (1995) Simultaneous analysis of saponins in ginseng radix by high performance liquid chromatography. Chem Pharm Bull 43:137-141

Seo H-S, Song J-T, Cheong J-J, Lee Y-H, Lee Y-W, Hwang I, Lee J-S, Choi Y-D (2001) Jasmonic acid carboxyl methyltransferase: A key enzyme for jasmonate-regulated plant responses. Proc Natl Acad Sci USA 98:4788-4793

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

Sohn H-B, Lee H-Y, Seo J-S, Jung C, Jeon J-H, Kim J-H, Lee Y-W, Lee J-S, Cheong J-J, Choi Y-D (2011) Overexpression of jasmonic acid carboxyl methyltransferase increases tuber yield and size in transgenic potato. Plant Biotechnol Rep 5:27-34

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

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

Wu J, Zhong JJ (1999) Production of ginseng and its bioactive components in plant cell culture:current technological and applied aspects. J Biotechnol 19:89-99

Yukimune Y, Tabata H, Higashi Y, Hara Y (1996) Methyl jasmonate-induced overproduction of paclitaxel and baccatin III in Taxus cell suspension cultures. Nat Biotechnol 14:1129-1132

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