Research article

Investigation of ginsenosides in different tissues after elicitor treatment in *Panax ginseng*

Ji Yeon Oh [☆], Yu-Jin Kim [☆], Moon-Gi Jang, Sung Chul Joo, Woo-Saeng Kwon, Se-Yeong Kim, Seok-Kyu Jung, Deok-Chun Yang[*]

Department of Oriental Medicinal Materials and Processing, College of Life Science, Kyung Hee University, Suwon, Korea

ARTICLE INFO

ABSTRACT

Background: The effect of methyl jasmonate (MJ) on ginsenoside production in different organs of ginseng (*Panax ginseng* Meyer) was evaluated after the whole plant was dipped in an MJ-containing solution. MJ can induce the production of antioxidant defense genes and secondary metabolites in plants. In ginseng, MJ treatment in adventitious root resulted in the increase of *dammarenediol synthase* expression but a decrease of *cycloartenol synthase* expression, thereby enhancing ginsenoside biosynthesis. Although a previous study focused on the application of MJ to affect ginsenoside production in adventitious roots, we conducted our research on entire plants by evaluating the effect of exogenous MJ on ginsenoside production with the aim of obtaining new approaches to study ginsenoside biosynthesis response to MJ *in vivo*.
Methods: Different parts of MJ-treated ginseng plants were analyzed for ginsenoside contents (fine root, root body, epidermis, rhizome, stem, and leaf) by high-performance liquid chromatography.
Results: The total ginsenoside content of the ginseng root significantly increased after 2 d of MJ treatment compared with the control not subjected to MJ. Our results revealed that MJ treatment enhances ginsenoside production not in the epidermis but in the stele of the ginseng root, implying transportation of ginsenosides from the root vasculature to the epidermis. Application of MJ enhanced protopanaxadiol (PPD)-type ginsenosides, whereas chilling treatment induced protopanaxatriol (PPT)-type ginsenosides.
Conclusion: These findings indicate that the production of PPD-type and PPT-type ginsenosides is differently affected by abiotic and biotic stresses in the ginseng plant, and they might play different defense mechanism roles.

1. Introduction

Panax ginseng Meyer, which is commonly known as Korean ginseng, is one of the most important traditional medicines in East Asia. Triterpene glycoside saponin, named ginsenoside, is the main bioactive ingredient in *P. ginseng* and is known to exhibit various pharmacological and physiological effects including anticancer [1–3], antidiabetic [4,5], immunomodulatory [1,6], neuroprotective [1], radioprotective [7], antiamnestic [1], and antistress properties [8,9]. The natural role of saponins in plants has been suggested to play a defensive role against pathogen and pest attacks [10]. The most important physiological role of ginsenosides in the ginseng plant is part of the defense mechanisms from pathogen attacks [11]. Naturally occurring ginsenosides are present to protect the ginseng from microbial and fungal infection; the bitter taste of ginsenosides makes them antifeedants [12–16].

Ginsenoside is contained in ginseng root at >4% by dry weight [17]. Ginsenosides are classified into two groups by the skeleton of aglycones, namely dammarane type and oleanane type. Dammarane-type tetracyclic structure is unique in ginseng, although other oleanane-type triterpenes are also observed in other plants. Dammarane-type ginsenosides consist mainly of two types that are classified according to their aglycone moieties, protopanaxadiol (PPD) and protopanaxatriol (PPT) ginsenoside.

* Corresponding author. Kyung Hee University, 1 Seocheon-dong, Kiheung-gu Yongin, Kyunggi-do 446-701, Korea.
  E-mail address: dcyang@khu.ac.kr (D.-C. Yang).
☆ These authors contributed equally to this work.

Ginsenoside backbones are synthesized via the isoprenoid pathway by cyclization of 2,3-oxidosqualene mediated by dammarenediol synthase (DDS) or β-amyrin synthase (β-AS). Although many reports have been published regarding the pharmacological effects of ginsenosides, little is known about the ginsenoside biosynthesis pathway or its regulation. Complete cDNA clones for several enzymes from ginseng have been reported. The genes encoding squalene synthase (SS), squalene epoxidase (SE), β-AS, lanosterol synthase, cycloartenol synthase (CAS), and DDS have been identified. Metabolic engineering such as overexpression or gene silencing of those genes has altered ginsenoside levels. Upregulation of ginsenoside levels by elicitors is also an attractive strategy to achieve greater ginsenoside quantities [18].

The accumulation of secondary metabolites can be enhanced by exposing plant cell and tissue cultures to biotic and abiotic elicitors [19]. When plants perceive environmental changes, they generate biological responses through specific signal transduction. Methyl jasmonate (MJ) has been reported to play an important role in the production of antioxidant defense genes and secondary metabolites in plants [20–22]. It has been reported that MJ stimulates ginsenoside production in cultured ginseng cells, hairy root, and adventitious roots [23–26].

MJ also increases the production of soyasaponin in cultured *Glycyrrhiza glabra* cells [27] and saikosaponin in the adventitious roots of *Bupleurum falcatum* [28]. The stimulation of saponin production by MJ treatment may be mediated by the upregulation of the genes involved in the biosynthesis of these saponins.

Elicitation using MJ treatment has been conducted on ginseng hairy roots and adventitious roots. Treatment of *in vitro* cultures with MJ may increase the production of ginsenosides up to ninefold [29]. However, no elicitation studies with MJ have been done with the entire *P. ginseng* plant. Although ginseng root is usually used for medicinal purposes, ginsenosides are distributed in many parts of the ginseng plant, including the root, leaf, and berry. Different parts of the plant contain distinct ginsenoside profiles [2], which may exhibit different pharmacological activities. We conducted our research on whole 3-yr-old ginseng plants. The aim of the present study was to investigate which organs of the ginseng plant respond to elicitor treatment *in vivo*, thereby potentially enhancing ginsenoside production.

2. Materials and methods

2.1. Ginseng materials and treatment

Three-yr-old ginseng plants hydroponically cultured in perlite and peat moss at $23 \pm 2°C$ under white fluorescent light ($60-100$ μmol/m^2/s) in a controlled greenhouse (kindly provided by i-farm in Yeo-Ju, Korea) were used for whole plant treatment. Ginseng roots were dipped in water containing 50μM MJ and were maintained in the dark. After 2 d, fine root, root body (the inner part including xylem and pith), epidermis (the outer surface including cortex), rhizome, stem, and leaf parts were separately used for ginsenoside analysis. For chilling treatment, 1-yr-old ginseng roots were kept at 4°C for 4 wk. For
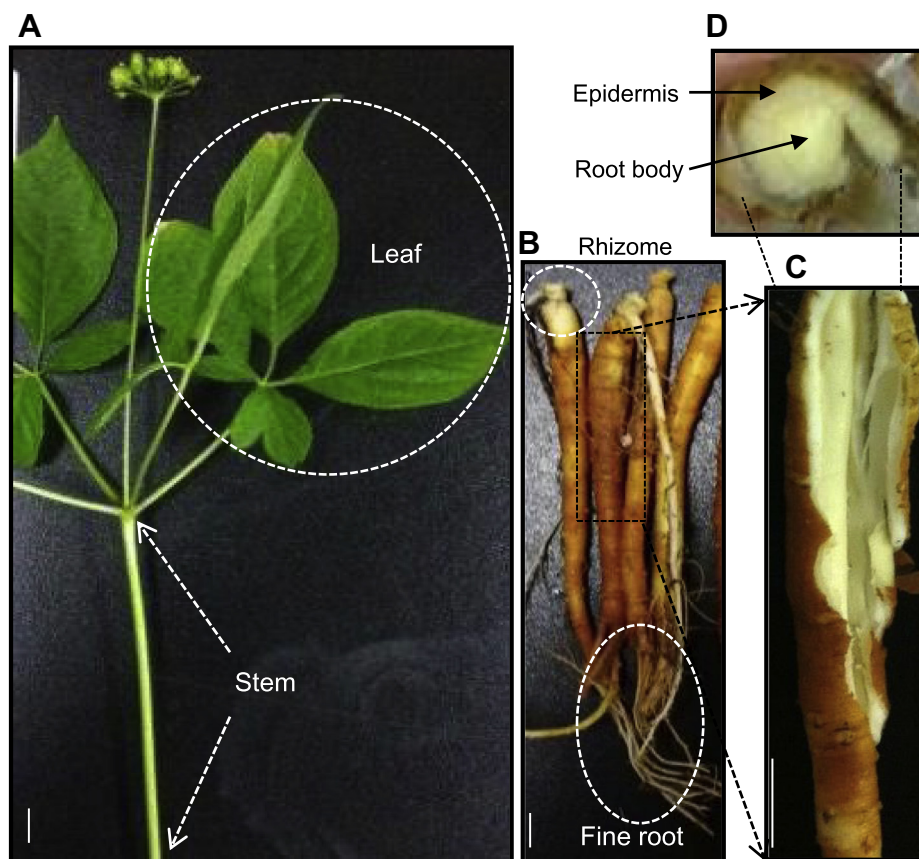

Fig. 1. Photographs of different organs of a 3-yr-old ginseng plant used for ginsenoside analysis. Three-yr-old ginseng plants hydroponically cultured in perlite and peat moss were used for methyl jasmonate (MJ) treatment. For ginsenoside analysis, different organs were sampled separately: the (A) leaf, stem, (B) rhizome, and fine root were sampled. The main root was divided again into the epidermis (the outer surface including the cortex) and (C) the root body (the inner part including the xylem and pith) by peeling. (D) A horizontal close-up image of the main root. All bars indicate 1 cm.

ginsenoside analysis, rhizome, epidermis, upper and lower root body, and fine root parts were sampled separately.

2.2. Analysis of ginsenosides by high-performance liquid chromatography

Milled powder (0.3−1 g) of freeze-dried adventitious roots, leaves, and roots of ginseng were twice soaked in an 80% (v/v) methanol solution at 70°C for 1 h. The extract was filtered and then evaporated to remove the liquid. The residue was dissolved in distilled water followed by extraction with water-saturated n-butanol. The butanol layer was then evaporated to produce the saponin fraction. Each sample was dissolved in methanol (1 g/5 mL), filtered using a 0.45-μm filter, and then used for high-performance liquid chromatography (HPLC) analysis. The HPLC separation was carried out on an Agilent 1260 series HPLC system (Palo Alto, CA, USA). This experiment employed a C18 (250 mm × 4.6 mm, ID 5 μm) column using distilled water (Solvent A) and acetonitrile (Solvent B) mobile phases, with a flow rate of 1.6 mL/min and the following gradient: A/B ratios of 80.5:19.5 for 0−29 min, 70:30 for 29−36 min, 68:32 for 36−45 min, 66:34 for 45−47 min, 64.5:35.5 for 47−49 min, 0:100 for 49−61 min, and 80.5:19.5 for 61−66 min. The sample was detected at a wavelength of 203 nm. Quantitative analysis was performed via a one-point curve method using external standards of authentic ginsenosides.

2.3. Statistical analyses

The values of the ginsenoside contents and relative gene expression were expressed as mean ± standard deviation (SD). Statistical analyses were carried out using the GraphPad Prism software (GraphPad, San Diego, CA, USA) by one-way analysis of variance (ANOVA). Duncan's multiple range test was employed to test for significant differences between the treatments at $p < 0.05$ and $p < 0.01$.

3. Results

3.1. Response of different ginseng tissues to MJ treatment

The total ginsenoside contents in each tissue of the entire ginseng plant were analyzed. Cultivation of ginseng by hydroponics involves a shorter cultivation period in a greenhouse in which variables such as light, temperature, moisture, and carbon dioxide content can be controlled [30,31]. Therefore, we used hydroponically cultured 3-yr-old ginseng plants (Fig. 1). Fig. 2 shows that ginsenoside accumulations within the aerial parts (leaf and stem) were increased as compared with the control. Total ginsenoside contents in the leaf were higher than other tissues. In addition, total ginsenoside contents within the underground parts (rhizome, root body, epidermis, and fine root) were also increased, except in the epidermis. Total ginsenoside contents of the root body in MJ-treated plants increased by approximately twofold compared with that of the control. This result demonstrates that the increase in ginsenoside contents of the root body is the highest among all tested ginseng organs. In rhizome, total ginsenoside accumulation and its composition was significantly increased after MJ treatment. Total ginsenoside content of fine roots was increased by approximately 6 mg/g compared with the control, which is the most increased content observed in underground parts. In the epidermis, total ginsenoside content was only minimally influenced by MJ treatment.

Fig. 3 shows the accumulation of individual ginsenosides in different tissues. The content of ginsenoside Re in aerial parts (leaf and stem) of the ginseng plant was the highest. In leaf, ginsenoside
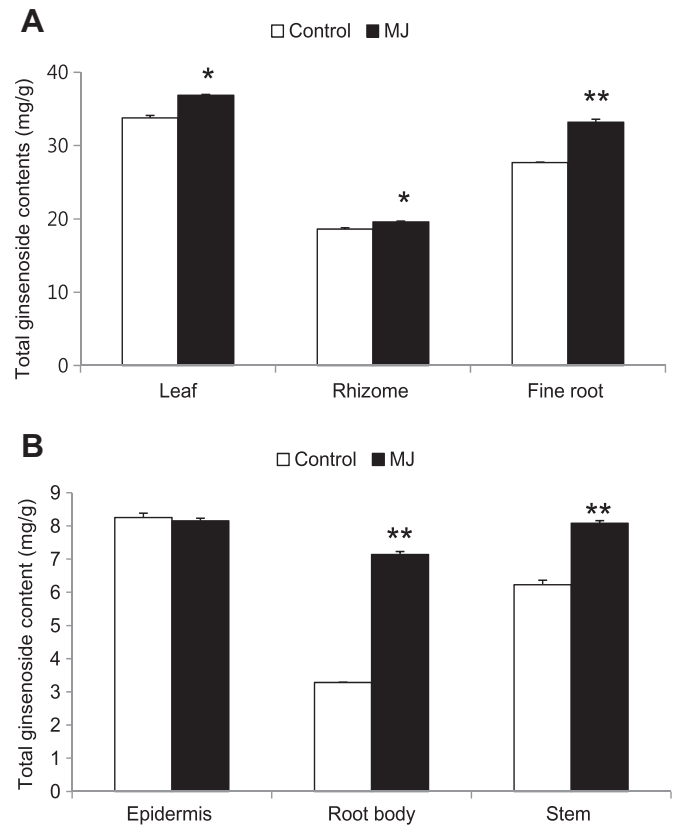

Fig. 2. Effects of methyl jasmonate (MJ) on ginsenoside production in different ginseng plant tissues. Three-yr-old ginseng plants cultured hydroponically in perlite and peat moss were treated with 50μM of MJ by dipping the root in a foil-wrapped glass vial for 2 d. Total ginsenoside contents in (A) the leaf, rhizome, and fine root, as well as (B) the epidermis, root body, and stem, shown in Fig. 1, were analyzed. Vertical bars indicate the mean value ± standard deviation from three independent experiments. *$p < 0.05$ and **$p < 0.01$ denote significant difference from the control, respectively.

Re and Rd contents were mainly enhanced. The ratio between PPD-type and PPT-type ginsenosides was significantly changed in the stem. The content of ginsenoside Rd was increased more than other ginsenosides; therefore, the ratio of PPD-type ginsenoside was increased. In rhizome, the ratio of PPD-type ginsenoside was also increased due to accumulated ginsenoside Rd, although the content of ginsenoside Rg1 in the rhizome was the highest. The greatest increase of ginsenoside level was shown in the root body. All individual ginsenoside contents were increased. Levels of ginsenosides Rb1 and Rg1 were doubled as compared with the control. Although the content of ginsenoside Rg1 was the highest, ginsenoside Rd was enhanced fivefold. In addition, ginsenoside Rc and Rb2, which was not detected in the control, accumulated after MJ treatment, showing in the increased ratio of PPD-type ginsenoside. In fine root, all individual ginsenosides were also increased. Fine roots contained mostly ginsenoside Re, but the ratio of ginsenoside Rb1 was enhanced upon MJ treatment. Therefore, the ratio of PPD-type ginsenoside was increased to greater than that of the PPT-type ginsenoside. In the epidermis, all individual ginsenoside accumulation was similar to the control.

3.2. Response of different ginseng tissues to chilling treatment

As a representative treatment for abiotic stress, we treated chilling stress to root and analyzed ginsenoside contents. Total ginsenoside contents of chilled ginseng were analyzed in different organs from 1-yr-old root (rhizome, epidermis, upper root body, lower
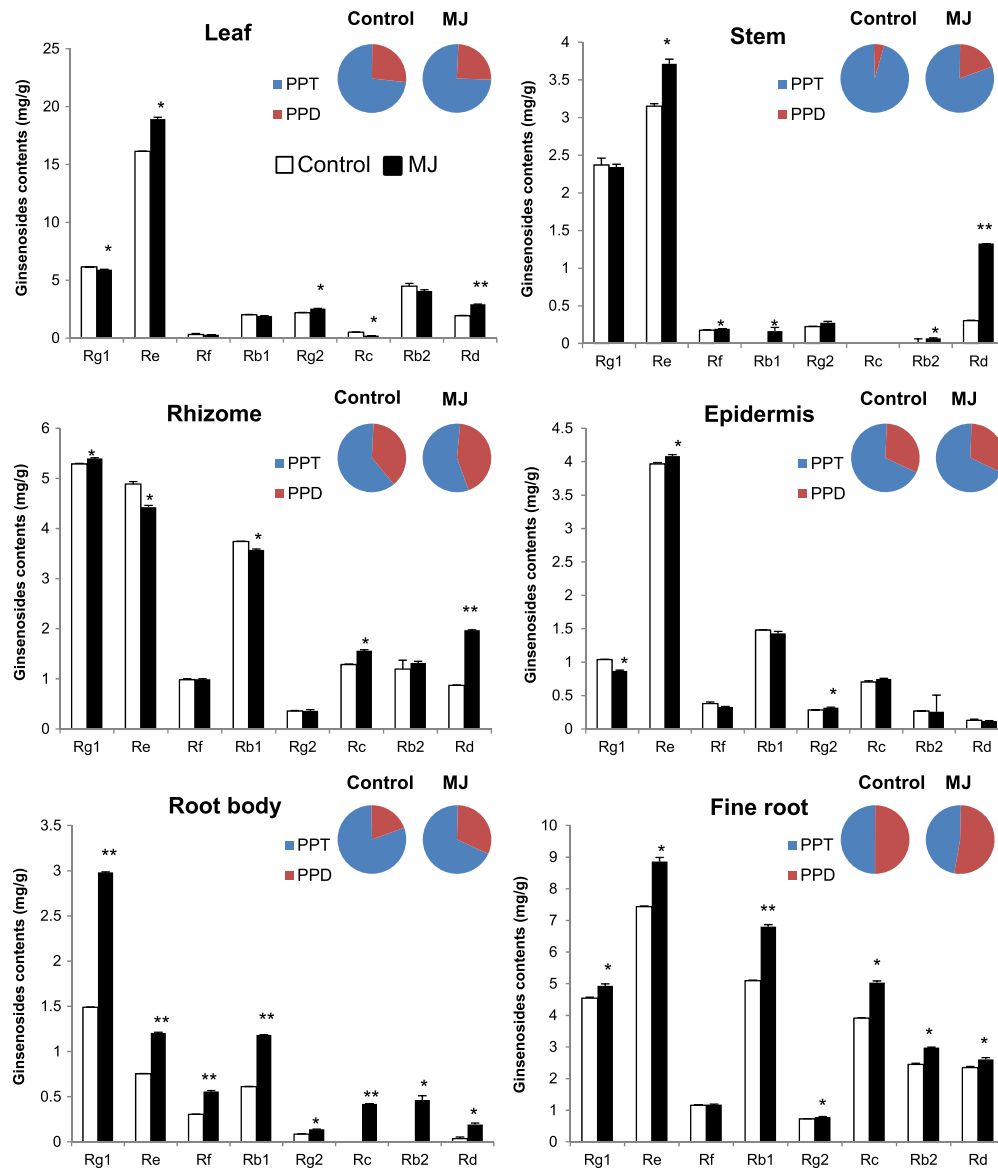
Fig. 3. Individual ginsenoside contents by methyl jasmonate (MJ) elicitation in different ginseng plant tissues. Three-yr-old ginseng plants were treated with 50μM of MJ for 2 d. Individual ginsenoside contents in the leaf, stem, rhizome, epidermis, root body, and fine root were analyzed. Protopanaxadiol (PPD) indicates the sum of ginsenoside Rb1, Rb2, Rc, and Rd, and protopanaxatriol (PPT) indicates the sum of ginsenoside Re, Rg, Rg1, and Rg2. Vertical bars indicate the mean value $\pm$ standard deviation from three independent experiments. $*p < 0.05$ and $**p < 0.01$ denote significant difference from the control, respectively.

root body, and fine root; Fig. 4). As shown in Fig. 4, total ginsenoside levels of all tissues were increased. In particular, total ginsenoside contents of the lower root body after removing the epidermis increased approximately eightfold as compared with the control. Total ginsenoside contents of the lower root body showed the highest increased level of approximately 14 mg/g compared with the control (Fig. 4C). Total ginsenoside contents of the upper root body after removing the epidermis were increased threefold as compared with the control. The ratio of ginsenoside accumulation in the upper root body to lower root body in the control was 1:2. After chilling treatment, this ratio was changed to 1:5. In addition, the ratio of ginsenoside accumulation in the lower root body to fine root in the control was 1:13. After chilling treatment, a 1:2 ratio was noted.

We also analyzed the contents of individual ginsenosides in different organs upon chilling treatment (Fig. 5). In the epidermis, the contents of ginsenosides Re, Rb1, and Rg2 were significantly increased after chilling. By contrast, ginsenoside Rg1 was decreased

in root rhizome and epidermis. Ginsenoside Re increased to the highest level in the epidermis as compared with the control ($\sim$6.6 mg/g) and was not significantly increased in rhizome (Fig. 5). In fine root, ginsenoside Re content was increased, whereas ginsenoside Rg1 content was essentially unchanged, and ginsenosides Rb1, Rc, and Rb2 were reduced. Ginsenoside Re content was increased to the highest ratio and level compared with the control in fine root. The upper and lower roots of the body both showed increased ginsenoside accumulation of most ginsenosides. In the upper root, ginsenosides Rc and Rb2 were not detected but were present after chilling treatment. Ginsenoside Rd content significantly increased by approximately 14-fold. All individual ginsenosides in the lower root body highly increased after chilling treatment (Fig. 5). Ginsenosides Re, Rb2, and Rd were dramatically enhanced. The ratio of ginsenosides Re and Rb2 was increased more than 20-fold. The ratio of PPT-type ginsenosides was increased in all tissues except the rhizome, which showed a static level.
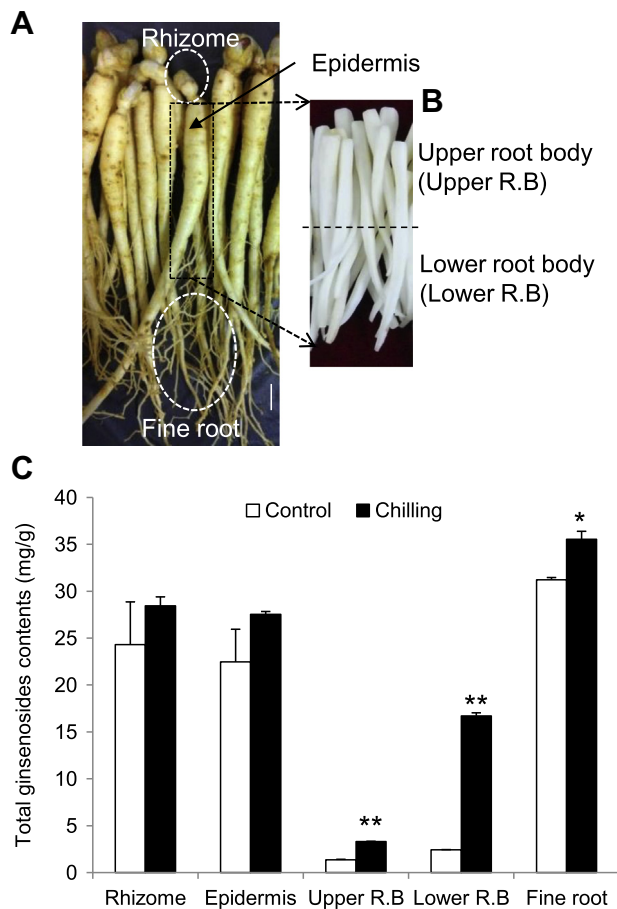
A

Rhizome

Epidermis

B

Upper root body
(Upper R.B)

Lower root body
(Lower R.B)

Fine root

C

Total ginsenosides contents (mg/g)

□ Control ■ Chilling

Rhizome   Epidermis   Upper R.B   Lower R.B   Fine root

Fig. 4. Effect of chilling on ginsenoside production in different ginseng plant tissues. (A) Photograph of different organs of a 1-yr-old ginseng root used for ginsenoside analysis. For ginsenoside analysis, different tissues were sampled separately; the rhizome and fine root were sampled. (B) After the epidermis was collected by peeling, the main root body was divided again into upper and lower halves. All bars indicate 1 cm. (C) Total ginsenoside contents in rhizome, epidermis, upper root body, lower root body, and fine root, shown in (A) and (B), were analyzed. Vertical bars indicate the mean value ± standard deviation from three independent experiments. *$p < 0.05$ and **$p < 0.01$ denote significant difference from the control, respectively.

4. Discussion

The roots of *P. ginseng* are used as important components of traditional oriental medicine [32], and the ginsenoside content increases with plant age [33]. Therefore, knowing where the ginsenosides localize in the ginseng root is important. Ginsenoside was reported to be at a higher content in the epidermis than in the cortex and xylem of the ginseng root [34]. Histochemical studies showed that ginsenosides are located in the periderm and outer cortex regions of the root, and root hairs contain higher ginsenoside contents than main roots [35,36]. *Panax quinquefolius* ginsenosides are also mostly detected in the periderm and cortex of the root [37]. Recently, multicenter matrix-assisted laser desorption/ionization mass spectrometry imaging confirmed that ginsenosides were more highly concentrated in the cortex and the periderm than that in the medulla of a lateral root, and localization of ginsenosides in the root tip is higher than that in the pith of the root [38]. In addition, a quantitative difference was detected between localizations of PPD-type ginsenosides (Rb1, Rb2, or Rc) and the PPT-type ginsenoside (Rf) in the root [38]. As saponins are known to be distributed to the root epidermis [34], we confirmed the accumulation of ginsenosides in the epidermis rather than the root body

(J. Y. Oh et al, unpublished). However, our data in other work showed that ginsenoside biosynthesis genes are expressed in the root vasculature, such as phloem [18,39]. This controversial distribution and biosynthesis information led us to hypothesize that ginsenosides are produced in the root vasculature and then transported to the epidermis for a defensive role. To confirm this hypothesis, we selected MJ, known as a strong effective elicitor, to stimulate the biosynthesis of ginsenoside *in vivo*.

To improve the metabolite contents, some elicitors have been used to increase the expression and activities of key enzymes in the rate-limiting step of the biosynthetic pathway. MJ is a key signaling compound involved in the elicitation process, which leads to the accumulation of secondary metabolites [40]. Because ginsenosides are secondary metabolites in ginseng, the accumulation of these compounds is also controlled by the treatment of elicitors such as MJ and salicylic acid [41,42]. The total ginsenoside content increases approximately fourfold following MJ treatment in suspension cultured adventitious roots [6]. Depending on the timing of MJ application, the adventitious roots appear to show different growth effects and ginsenoside production. It was shown from previous reports that the addition of MJ at the early phase of *P. ginseng* growth inhibits adventitious root growth [43]. Jasmonic acid (JA) also strongly inhibited ginseng hairy root growth. [23]. To prevent a reduced biomass of adventitious roots, mostly 10μM of MJ was used in adventitious root cultures of *P. ginseng* 4 wk after inoculation. Higher MJ concentration and extended cultivation time also showed effects on root growth [43,44].

According to previous studies, this elicitation effect of ginsenosides is attributable to an MJ-induced expression of ginsenoside biosynthetic genes [6,29]. In our experiment using ginseng adventitious root, the expression of *PgHMGR1*, which plays a role in the production of mevalonic acid (MVA), was increased threefold (J. Y. Oh et al, unpublished). The expression of *PgDDS*, which is involved in the dammarenediol backbone for ginsenoside, was strongly upregulated, whereas *PgCAS* was decreased (data not shown). This expression pattern is similar to a previous report wherein MJ induced changes of triterpene saponins in ginseng hairy root [29]. Exposure to MJ at 100μM in hairy roots of *P. ginseng* induced the expression of genes involved in ginsenoside biosynthesis, such as *PgSS*, *PgSE*, and *PgDDS*, with a slight decrease of *PgCAS* [29,45], suggesting that MJ as a signal transducer may stimulate ginsenoside production by activation of the enzymes in the MVA pathway to dammarenediol and may also inactivate enzymes for sterol production.

Our present and previous results confirmed MJ as an effective elicitor of ginsenoside synthesis in ginseng adventitious roots. However, until now, it was not clear if MJ had the same effect on the whole ginseng plant. In this study, we tested the effect of MJ as an elicitor of ginsenoside accumulation in whole ginseng plants. When we analyzed the ginsenoside contents of the whole ginseng plant after exposing the ginseng root to MJ for 2 d, a pronounced increase of the ginsenosides was observed in the leaf, stem, root body, and fine root, with the greatest increase noted in the root body. An interesting observation was that most accumulation was observed in the root body, not the epidermis, which is known to have a high ginsenoside content. Rather, the epidermis did not show any alteration, indicating that ginsenoside biosynthesis actively occurs in the root body. After production in the root cortex, ginsenosides may be transported to the epidermis to play a defensive role. Ginsenosides can be synthesized in vasculature tissue such as phloem [46] and then be transported for storage or play a defensive role. Saponin glycosides can be stored in vacuoles through the fusion of endoplasmic reticulum-derived vesicles [47] or transported by the ATP-binding cassette transporter [48] or multidrug and toxic compound extrusion transporters [49,50]. Further studies
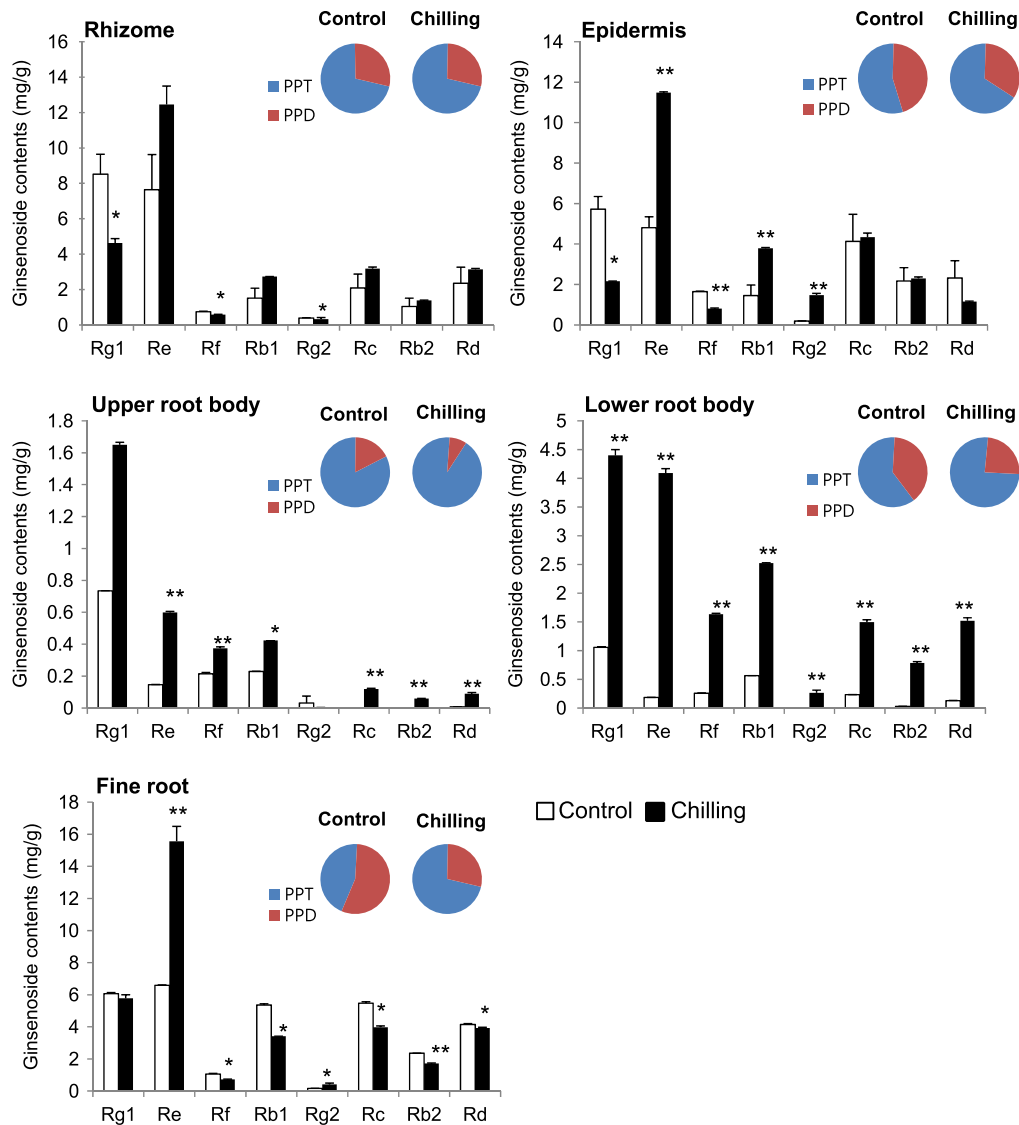
Fig. 5. Individual ginsenoside contents upon chilling stress treatment in different ginseng plant tissues. One-yr-old ginseng plants were subjected to chilling for 4 wk. Individual ginsenoside contents in rhizome, epidermis, upper root body, lower root body, and fine root, as shown in Fig. 4, were analyzed. Protopanaxadiol (PPD) indicates the sum of ginsenoside Rb1, Rb2, Rc, and Rd, and protopanaxatriol (PPT) indicates the sum of ginsenoside Re, Rg, Rg1, and Rg2. Vertical bars indicate the mean value ± standard deviation from three independent experiments. *$p < 0.05$ and **$p < 0.01$ denote significant difference from the control, respectively.

on the ginsenoside transporter will provide more detail regarding ginsenoside transport.

Upon exposure of hairy roots or roots of *P. ginseng* to MJ, both tissues showed increased PPD-type ginsenoside content, whereas PPT-type ginsenoside content changed only slightly compared with controls [6,29]. JA also improved the accumulation of PPD ginsenosides much more than PPT ginsenosides [23], indicating that JA and its MJ elicitor might have triggered the synthesis of PPD-type ginsenosides. Similarly, different tissues in our experiment showed more accumulation of PPD-type ginsenosides, especially in the stem, root body, and fine root (Fig. 3). The recent discovery of *protopanaxadiol synthase* (*PPDS*), a *cytochrome P450* (*P450*) for production of PPD, confirmed its induction by MJ treatment [51]. However another *P450* which is involved in PPT production from PPD, named *protopanaxatriol synthase* (*PPTS*), does not respond to MJ treatment [52]. This suggests that MJ more highly stimulates the pathway leading to PPD ginsenoside synthesis than PPT ginsenoside synthesis. Although the biosynthetic pathway of dammarenediol into different ginsenosides has yet to be determined,

further studies for identification of glycosyl transferases, enzymes in biosynthetic steps from PPD or PPT to different individual ginsenosides, will elucidate the different synthesis mechanisms of individual ginsenosides.

Overall, PPT-type ginsenoside accumulation was enhanced by chilling treatment (Fig. 5). When we performed chilling treatment as an abiotic stress, PPT-type ginsenosides were increased in the epidermis, upper and lower root body, and fine root, differently than with MJ treatment. This implies that ginseng roots are capable of differentially activating distinct defense pathways. The production of various secondary metabolites, including ginsenosides, is usually associated with defense responses to stresses [53]. JA and its methyl ester MJ are signaling compounds that modulate various physiological processes in plants, such as root growth, senescence, and the defense response against pathogens and insect attack [54]. In addition, MJ induces or increases the biosynthesis of many secondary metabolites that play important roles in the adaptation of plants to particular environments [21,55]. MJ treatment actually mimics a pathogen or herbivorous attack [56]. Saponins also have
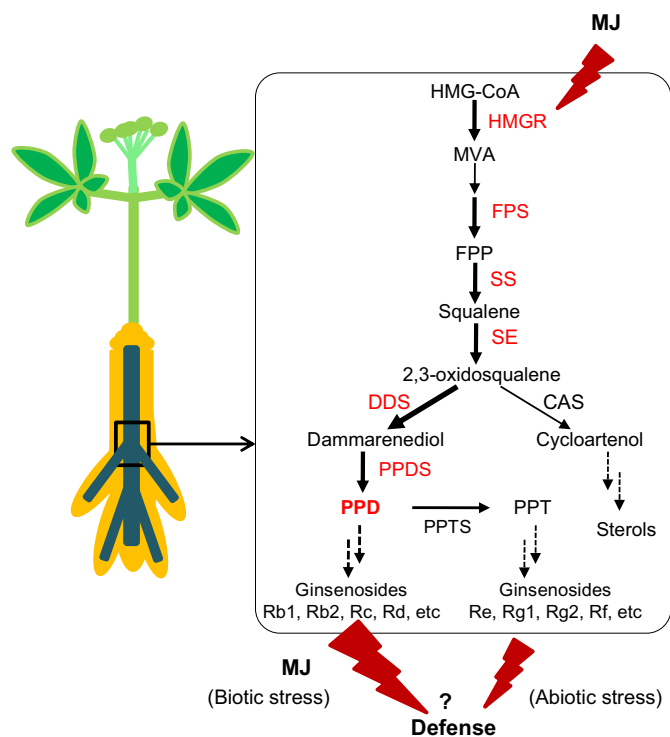
Fig. 6. Summary of ginsenoside biosynthesis in ginseng root by elicitation. Ginsenoside production is stimulated via mevalonate (MVA) pathway genes, which are upregulated by MJ signaling. MJ elicitation enhanced PPD-type ginsenosides, whereas chilling stress increased PPT-type ginsenosides. CAS, cycloartenol synthase; DDS, dammarenediol synthase; FPP, farnesyl diphosphate; FPS, farnesyl diphosphate synthase; HMGR, 3-hydroxy-3-methylglutaryl-CoA reductase; MJ, methyl jasmonate; MVA, mevalonic acid; PPD, protopanaxadiol; PPDS, PPD synthase (cytochrome P450); PPT, protopanaxatriol; PPTS, PPT synthase (cytochrome P450); SE, squalene epoxidase; SS, squalene synthase.

potent antifungal activities, as has been shown for avenacins, which are oleanane-type triterpene saponins found in oats [24]. However, although there is less known about the physiological role of ginsenoside, it is also considered to have a defensive role [12]. Different elicitation effects of MJ and chilling on individual ginsenosides might be explained by the differentiated defense strategy of ginsenosides (Fig. 6), correlated with the different biological activities of different ginsenosides.

To the best of our knowledge, this study is the first to report on the evaluation of individual ginsenoside levels in separate epidermis and root body. Moreover, this is the first study to provide a detailed profile of ginsenoside composition in different organs of whole ginseng plants following elicitor treatment, although further studies of gene expression in different tissues of ginseng will be required.

Conflict of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

References

[1] Attele AS, Wu JA, Yuan CS. Ginseng pharmacology: multiple constituents and multiple actions. Biochem Pharmacol 1999;58:1685–93.

[2] Im KS, Chung HY, Park SH, Je NK. Anticancer effect of the hydrolyzed mono-gluco-ginsenoside of total saponin from ginseng leaf. J Ginseng Sci 1995;19:291–4.

[3] Li X, Wan HG, Lu DQ, Wei P. Advance of research on antitumour activity of ginsenosides. Chin J Bioproc Eng 2003;1:13–7.

[4] Xie JT, Mehendale SR, Li X, Quigg R, Wang X, Wang CZ, Wu JA, Aung HH, Rue PA, Bell GI, et al. Anti-diabetic effect of ginsenoside Re in ob/ob mice. Biochem Biophys Acta 2005;1740:319–25.

[5] Shang W, Yang Y, Zhou L, Jiang B, Jin H, Chen M. Ginsenoside Rb1 stimulates glucose uptake through insulin-like signaling pathway in 3T3-L1 adipocytes. J Endocrinol 2008;198:561–9.

[6] Wang J, Gao WY, Zhang J, Zuo BM, Zhang LM, Huang LZ. Advances in study of ginsenoside biosynthesis pathway in *Panax ginseng* C. A. Meyer. Acta Physiol Plant 2012;34:397–403.

[7] Lee TK, Johnke RM, Allison RR, O'Brien KF, Dobbs LJ. Radio protective potential of ginseng. Mutagenesis 2005;20:237–43.

[8] Saito H, Yoshida Y, Takagi K. Effect of *Panax ginseng* root on exhaustive exercise in mice. Jpn J Pharmacol 1974;24:119–27.

[9] Gillis CN. *Panax ginseng* pharmacology: a nitric oxide link? Biochem Pharmacol 1997;54:1–8.

[10] Osbourn AE. Preformed antimicrobial compounds and plant defense against fungal attack. Plant Cell 1996;8:1821–31.

[11] Leung KW, Wong AS. Pharmacology of ginsenosides: a literature review. Chin Med 2010;5. article 20.

[12] Nicol RW, Traquair JA, Bernards MA. Ginsenosides as host resistance factors in American ginseng (*Panax quinquefolius*). Can J Bot 2002;80:557–62.

[13] Katerere DR, Gray AI, Nash RJ, Waigh RD. Antimicrobial activity of pentacyclic triterpenes isolated from African Combretaceae. Phytochemistry 2003;63:81–8.

[14] Mallvadhani UV, Mahapatra A, Raja SS, Manjula C. Antifeedant activity of some pentacyclic triterpene acids and their fatty acid ester analogues. J Agric Food Chem 2003;51:1952–5.

[15] Bernards MA, Yousef LF, Nicol RW. The allelopathic potential of ginsenosides. In: Inderjit, Mukerji KG, editors. Allelochemicals: biological control of plant pathogens and diseases. Dordrecht: Springer; 2006. pp. 157–75.

[16] Sung WS, Lee DG. In vitro candidacidal action of Korean red ginseng saponins against *Candida albicans*. Biol Pharm Bull 2008;31:139–43.

[17] Shibata S. Chemistry and cancer preventing activities of ginseng saponins and some related triterpenoid compounds. Kor J Med Crop Sci 2001;16:28–37.

[18] Han JY, In JK, Kwon YS, Choi YE. Regulation of ginsenoside and phytosterol biosynthesis by RNA interferences of squalene epoxidase gene in *Panax ginseng*. Phytochemistry 2010;71:36–46.

[19] Gundlach H, Muller MJ, Kutchan TM, Zenk MH. Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proc Natl Acad Sci USA 1992;89:2389–93.

[20] Hampel D, Mosandl A, Wust M. Induction of de novo volatile terpene biosynthesis via cytosolic and plastidial pathways by methyl jasmonate in foliage of *Vitis vinifera* L. J Agric Food Chem 2005;53:2652–7.

[21] Martin DM, Gershenzon J, Bohlmann J. Induction of volatile terpene biosynthesis and diurnal emission by methyl jasmonate in foliage of Norway spruce. Plant Physiol 2003;132:1586–99.

[22] Farmer EE, Ryan CA. Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proc Natl Acad Sci USA 1990;87:7713–6.

[23] Yu KW, Gao WY, Son SH, Paek KY. 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 2000;36:424–8.

[24] Hu WW, Yao H, Zhong JJ. Improvement of *Panax notoginseng* cells for production of ginseng saponin and polysaccharide by high cell density cultivation in pneumatically agitated bioreactors. Biotechnol Prog 2001;17:838–46.

[25] Han J, Zhong JJ. High density cell culture of *Panax notoginseng* for production of ginseng saponin and polysaccharide in an airlift bioreactor. Biotechnol Lett 2002;24:1927–30.

[26] Wang W, Zhang ZY, Zhong JJ. Enhancement of ginsenoside biosynthesis in high-density cultivation of *Panax notoginseng* cells by various strategies of methyl jasmonate elicitation. Appl Microbiol Biotechnol 2005;67:752–8.

[27] Hayashi H, Hung P, Inoue K. Up-regulation of soyasaponin biosynthesis by methyl jasmonate in cultured cells of *Glycyrrhiza glabra*. Plant Cell Physiol 2003;44:404–11.

[28] Aoyagi H, Kobayashi Y, Yamada K, Yokoyama M, Kusakari K, Tanaka H. Efficient production of saikosaponins in *Bupleurum falcatum* root fragments combined with signal transducers. Appl Microbiol Biotechnol 2001;57:482–8.

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

[30] Kim GS, Lee SE, Noh HJ, Kwon H, Lee SW, Kim SY, Kim YB. Effects of natural bioactive products on the growth and ginsenoside contents of *Panax ginseng* cultured in an aeroponic system. J Ginseng Res 2012;36:430–41.

[31] Lee GA, Chang YK, Park SY, Kim GA, Kim SH, Park KC, Kim YB, Cha SW, Song BH. Comparative analysis on concentration and uptake amount of mineral nutrients in different growth stages and temperatures of *Panax ginseng* C. A. Meyer grown with hydroponic culture. Kor J Med Crop Sci 2012;20:251–8.

[32] Lee OR, Sathiyara G, Kim YJ, In JG, Kwon WS, Kim JH, Yang DC. Defense genes induced by pathogens and abiotic stresses in *Panax ginseng* C. A. Meyer. J Ginseng Res 2011;35:1–11.

[33] Shi W, Wang Y, Li J, Zhang H, Ding L. Investigation of ginsenosides in different parts and ages of *Panax ginseng*. Food Chem 2007;102:664–8.

[34] Kim MW, Ko SR, Choi KJ, Kim SC. Distribution of saponin in various sections of *Panax ginseng* root and changes of its contents according to root age. J Ginseng Res 1987;11:10–6.

[35] Tani T, Michinori K, Tadahisa K, Masayuki H, Teruaki H, Shigaru A, Histochemistry II. Ginsenoside in ginseng (*Panax ginseng*) root. J Nat Prod 1981;44:401–7.

[36] Christensen LP, Jensen M, Kidmose U. Simultaneous determination of ginsenosides and polyacetylenes in American ginseng root (*Panax quinquefolium* L.) by high performance liquid chromatography. J Agric Food Chem 2006;54:8995–9003.

[37] Smith RG, Caswell D, Carriere A, Zielke B. Variation in the ginsenoside content of American ginseng, *Panax quinquefolius* L., roots. Can J Bot 1995;74:1616–20.

[38] Taira S, Ikeda R, Yokota N, Osaka I, Sakamoto M, Kato M, Sahashi Y. Mass spectrometric imaging of ginsenosides localization in *Panax ginseng* root. Am J Chin Med 2010;38:485–93.

[39] Kim TD, Han JY, Huh GH, Choi YE. Expression and functional characterization of three squalene synthase genes associated with saponin biosynthesis in *Panax ginseng*. Plant Cell Physiol 2011;52:125–37.

[40] Hu X, Neill S, Cai W, Tang Z. Hydrogen peroxide and jasmonic acid mediate oligogalacturonic acid-induced saponin accumulation in suspension-cultured cells of *Panax ginseng*. Physiol Plant 2003;118:414–21.

[41] Ali MB, Yu KW, Hahn EJ, Paek KY. 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 2006;25:613–20.

[42] Wang J, Gao W, Zuo B, Zhang L, Huang L. Effect of methyl jasmonate on the ginsenoside content of *Panax ginseng* adventitious root cultures and on the genes involved in triterpene biosynthesis. Res Chem Interned 2012;39:1973–80.

[43] Kim YS, Hahn EJ, Murthy HN, Paek KY. Adventitious root growth and ginsenoside accumulation in *Panax ginseng* cultures as affected by methyl jasmonate. Biotechnol Lett 2004;26:1619–22.

[44] Yu KW, Gao W, Han EJ, Paek KY. Jasmonic acid improves ginsenoside accumulation in adventitious root culture of *Panax ginseng* C.A. Meyer. Biochem Eng 2002;11:211–5.

[45] Lee MH, Jeong JH, Seo JW, Shim CG, Kim YS, In JG, Yang DC, Yi JS, Choi YE. Enhanced triterpene and phytosterol biosynthesis in *Panax ginseng* over-expressing squalene synthase gene. Plant Cell Physiol 2004;45:976–84.

[46] Fukuda N, Shan S, Tanaka H, Shoyama Y. New staining methodology: Eastern blotting for glycosides in the field of Kampo medicines. J Nat Med 2006;60:21–7.

[47] Poustka F, Irani NG, Feller A, Lu Y, Pourcel L, Frame K, Grotewalk EA. Trafficking pathway for anthocyanins overlaps with the endoplasmic reticulum-to-vacuole protein sorting route in *Arabidopsis* and contributes to the formation of vacuolar inclusions. Plant Physiol 2007;145:1323–35.

[48] Jasiński M, Stukkens Y, Degand H, Purnelle B, Marchand-Brynaert J, Boutry M. A plant plasma membrane ATP binding cassette-type transporter is involved in antifungal terpenoid secretion. Plant Cell 2001;13:1095–107.

[49] Goodman CD, Casati P, Walbot YA. Multidrug resistance-associated protein involved in anthocyanin transport in *Zea mays*. Plant Cell 2004;16:1812–26.

[50] Yazaki K. Transporters of secondary metabolites. Curr Opin Plant Biol 2005;8:301–7.

[51] Han JY, Kim HJ, Kwon YS, Choi YE. The cyt P450 enzyme CYP716A47 catalyzes the formation of protopanaxadiol from dammarenediol-II during ginsenoside biosynthesis in *Panax ginseng*. Plant Cell Physiol 2011;52:2062–73.

[52] Han JY, Hwang HS, Choi SW, Kim HJ, Choi YE. Cytochrome P450 CYP716A53v2 catalyzes the formation of protopanaxatriol from protopanaxadiol during ginsenoside biosynthesis in *Panax ginseng*. Plant Cell Physiol 2012;53:1535–45.

[53] Zhao J, Davis LC, Verpoorte R. Elicitor signal transduction leading to production of plant secondary metabolites. Biotechnol Adv 2005;23:283–333.

[54] Wasternack C, Parthier B. Jasmonate-signaled plant gene expression. Trends Plant Sci 1997;2:302–7.

[55] Yukimine Y, Tabata H, Higashi Y, Hara Y. Methyl jasmonate-induced over-production of paclitaxel and baccation III in Taxus cell suspension cultures. Nat Biotechnol 1996;14:1129–32.

[56] Textor S, Gershenzon J. Herbivore induction of the glucosinolate-myrosinase defense system: major trends, biochemical bases and ecological significance. Phytochem Rev 2009;8:149–70.