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Research article

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

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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.

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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 ± 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

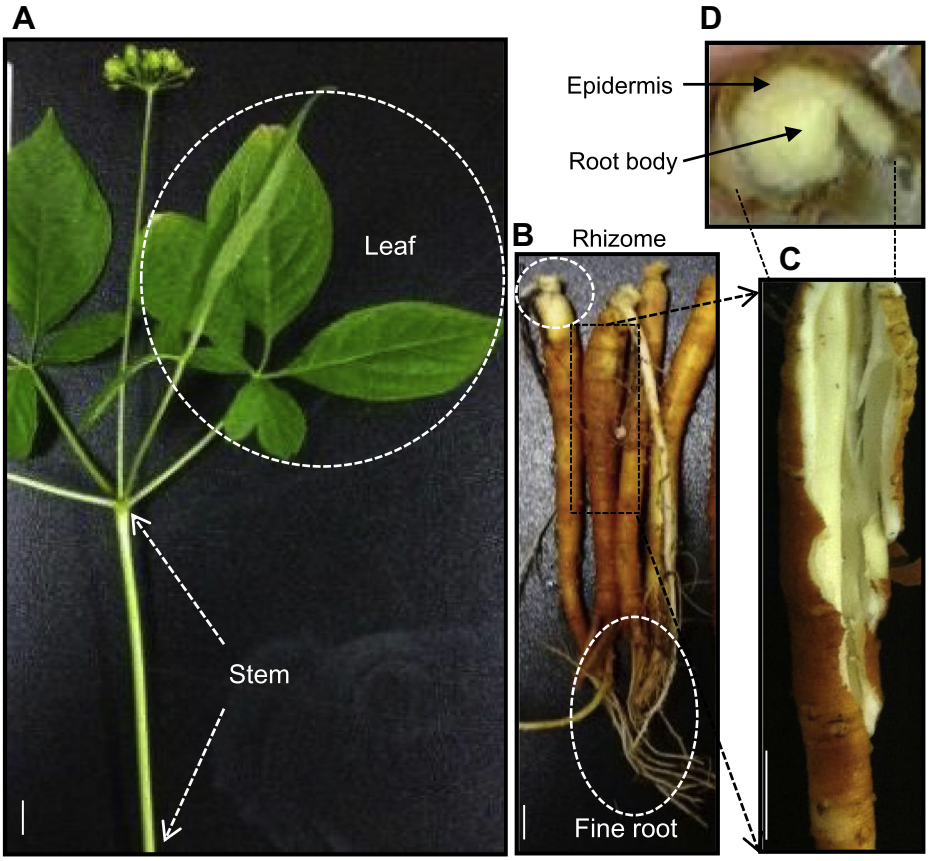


**Fig. 1.** Photographs of different organs of a 3-yr-old ginseng plant used for ginsenoside analysis. Three-yr-old ginseng plants hydroponically cultured in perlite and peat moss were used for methyl jasmonate (MJ) treatment. For ginsenoside analysis, different organs were sampled separately: the (A) 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.