Molecular characterization of ginseng farnesyl diphosphate synthase gene and its up-regulation by methyl jasmonate

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Abstract

We isolated a gene encoding for farnesyl diphosphate synthase (FPS) from *Panax ginseng*, a species that produces a large quantity of triterpene saponins such as ginsenosides. The deduced amino acid sequence of PgFPS was 77, 84 and 95 % identical to those of *Arabidopsis*, *Hevea*, and *Centella*. Southern blot analysis indicated that *P. ginseng* contained more than two genes encoding for FPS. When the cDNA of *PgFPS* was expressed in *Escherichia coli*, the recombinant enzyme, purified with a His-tag column, was found to possess FPS activity. When cultures of ginseng hairy root were treated with 0.1 mM methyl jasmonate (MJ), *PgFPS* mRNA was detected within 12 h of the treatment, and achieved maximum after 24 h. Also FPS activity in the hairy root cultures after 12 h of MJ treatment was higher than that of the control.

Additional key words: isoprenoid pathway, ginsenoside, squalene synthase, triterpene saponins.

Introduction

Farnesyl diphosphate synthase (FPS) is a pivotal enzyme in isoprenoid biosynthesis (Chappell 1995). The initial reaction of the isoprenoid pathway entails the conversion of three units of acetyl-CoA to 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA). The HMG-CoA is then reduced to mevalonate by 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGR). FPS catalyzes the sequential 1′-4 condensation of isopentenyl diphosphate (IPP) with dimethylallyl diphosphate (DMAPP) and then with the resultant geranyl diphosphate (GPP), yielding farnesyl diphosphate (FPP). Sesquiterpenes in plants are initially synthesized from FPP by sesquiterpene cyclases. In the triterpene pathway, two FPP molecules are converted by squalene synthase into squalene, the common precursor of both the sterols and other triterpenes. Not only is FPP a common precursor for sesquiterpene compounds (McCarvey and Croteau 1995), but is also the branching point of pathways resulting in the synthesis of a broad variety of triterpene products.

A number of FPSs have been reported in plants. A large body of information regarding FPS has been accumulated, and functions of the gene in *Arabipodsis thaliana* plants overexpressing FPS have been recently reported (Masferrer *et al*. 2002, Manzano *et al*. 2004). By overexpression of a chimeric FPS gene in transgenic plants, Chen *et al*. (2000) have demonstrated that FPS performs a regulatory function in sesquiterpene biosynthesis. However, no evidence for the regulatory role of FPS in triterpene saponins has yet been presented.

High ginsenoside production from *Panax ginseng* has been established by adventitious root cultures induced by γ-irradiation (Kim et al. 2009). On the other hand, methyl jasmonate (MJ) treatments as an elicitor have been previously applied in order to overproduce secondary metabolites in plants. These include triterpene saponins from cultured *Panax ginseng* cells (Lu *et al*. 2001), *Bupleurum falcatum* root fragments (Aoyagi *et al*. 2001), and *Centella asiatica* leaves of whole cultured plants (Kim *et al*. 2004b); sesquiterpene from *Hyoscyamus muticus* (Singh *et al*. 1998); and diterpene from *Taxus* (Kim *et al*. 2004a). Recently, Wolucka *et al*. (2005) suggested that MJ may stimulate vitamin C synthesis in *Arabidopsis* and tobacco cells *via de novo* biosynthesis.

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Abbreviations: DMAPP - dimethylallyl diphosphate; IPP - isopentenyl diphosphate; FPS - farnesyl diphosphate synthase; SQS - squalene synthase; GPP - geranyl diphosphate; MJ - methyl jasmonate; PgFPS - *Panax ginseng* farnesyl diphosphate synthase; PgSS - *Panax ginseng* squalene synthase.
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MJ can be considered as a crucial factor for studying secondary metabolites in ginseng and especially investing the level of gene expression associated with triterpene biosynthesis. Ginseng harbors a number of ginsenosides as triterpene saponins, and the roots harbor a large quantity of saponins. Total saponin conent is higher in the epidermis than in the cortex and xylem (Kim *et al*. 1987). Therefore, ginseng root is a favorable candidate for studies of triterpene biosynthesis and the regulation of those pathways *via* elicitor influences.

As an initial step in determining the regulatory mechanisms in the sesquiterpene and triterpene pathways, we isolated a gene encoding for FPS from ginseng roots, and characterized the gene using molecular techniques. Northern blot analysis was used to reveal the effects of methyl jasmonate (MJ) on the levels of ginseng *FPS* mRNA in the hairy roots, and FPS activity assays were performed on soluble extracts of the roots.

Materials and methods

Plants: 4-year-old *Panax ginseng* C.A. Meyer was provided by the National Institute of Crop Science (Suwon, Korea). The *P. ginseng* plants were harvested and immediately frozen with liquid nitrogen and stored at -80 °C. Total RNA was extracted from the roots. For MJ treatment experiments, a hairy root culture of *P. ginseng* was generated by infection with *Agrobacterium rhizogenes* strain A4, as previously reported (Hwang and Ko 1989). After 5 weeks of pre-cultivation, 0.1 mM MJ was added to the Murashige and Skoog (1962; MS) medium.

***P. ginseng* cDNA synthesis:** Total RNA was isolated from the *P. ginseng* roots using an RNeasy plant mini-kit (*Qiagen*, Hilden, Germany), according to the manufacturer's instructions. From the total RNA, mRNA was purified using a poly(A)+ RNA purification kit (*Qiagen*), and reverse-transcribed with oligo(dT) primer (RACE 32, 5′-GACTCGAGTCGACATCGATTTTTTT TTTTTTT-3′) (Frohman *et al*. 1988), and *SuperScript III* (MMLV) reverse transcriptase (*Invitrogen*, Carlsbad, USA) at 42 °C, in accordance with the manufacturer's instructions. The first-strand cDNA was then further treated with RNase H in order to digest the RNA strand attached to the first-strand cDNA.

Cloning of *FPS* cDNA: To amplify the core fragments, we utilized degenerate primers designed from highly conserved regions of *FPS* (Pan *et al*. 1996). The nucleotide sequences of these primers were as follows: F1 = 5′-TGYATHGARTGGYTICARGCITAYTTY-3′, R1 = 5′-RAARCARTCIARRTARTCRTCYTG-3′. PCR was first conducted for 30 cycles of 94 °C, 1 min, 45 °C, 2 min, and 72 °C, 3 min, and a final 10-min extension at 72 °C using a *PTC-200 Thermal Cycler* (*MJ Research*, Watertown, USA). A secondary PCR was conducted with the same primers as the first one and the first PCR product (0.005 cm^3) was used as a template, yielding a 520-bp fragment. The PCR products were recovered by electrophoresis on 1.5 % agarose gel, cloned into pGEM-T Easy vector (*Promega*, Madison, USA) and then transformed into competent *Escherichia coli* DH5α cells. Plasmid DNA was prepared from 10 individual transformants and sequenced using an automatic genetic analyzer *3100* (*Tropix*, *Applied Biosystem*, Forster City, USA).

Rapid amplification of cDNA ends: In order to characterize the open reading frame (ORF) of this *FPS*,5'-rapid amplification of cDNA ends (RACE) and 3'-RACE were conducted. For 5'-RACE, mRNA was reverse-transcribed with degenerate primer R1 to synthesize the 5' cDNA pool. The primer sequences were as follows: for *FPS*, 5′-RACE 5′-CCTTGGGATATG GTTGCGAAG-3′ and nested primer 5′-ACCTCT GCGCGTATGAGAGCTA-3′; 3′-RACE 5′-CCTATT CATCGCCGGATTGTG-3′ and the nested primer 5′-TCAGGCGAAGATCTGGAGAAAC-3′. PCR was conducted for 30 cycles of 94 °C, 1 min, 58 °C, 1 min, 72°C, 1 min, and a final 10-min extension at 72 °C. The first products were amplified using RACE32 primer, and the products purified by PCR purification kit (*Qiagen*) were used as templates for a second PCR with the nested primer and an anchor primer (5′-GACTCGAGT CGACATCGA-3′).

Construction of *PgFPS* expression vectors: The entire ORF region of the *PgFPS* cDNA was PCR amplified using forward primer (5′-ACAGAGCTCATGAGCG ATCTGAAGA-3′) (*Sac*I restriction site underlined and translation start codon in boldface), and reverse primer (5′-AATCTCGAGCTTTTGCCGCTTATAT-3′) harboring an *Xho*I site (underlined). The resulting 1044-bp PCR product (*PgFPS*) was digested with *Sac*I and *Xho*I, gel-purified, and ligated into the *Sac*I and *Xho*I sites in pET32a (*Novagen*, Madison, USA). Compared to the wild-type *PgFPS*, the recombinant fusion protein has a 159-amino acid stretch of Trx/His/S-tag at the N terminus, and the stop codon was eliminated *via* PCR amplification, in order to fuse it with a His-tag (LEHHHHHH) located at the C terminus. The resultant pET32-*PgFPS* was transformed into *E. coli* strain BL21 (DE3) pLysS.

***PgFPS* expression and FPS activity in *E. coli*:** Expression of the pET32a-*PgFPS* vector in *E. coli* and purification of the encoded His-tagged 56.1-kDa peptide was conducted *via* nickel affinity chromatography, as follows. A colony of BL21 (DE3) pLysS cells harboring the desired expression vector was cultured in 200 cm^3 culture medium at 37 °C. The culture was then grown to an absorbance of 0.5 at 600 nm, and then shifted to 28 °C,

after which isopropyl thio-β-D-galactoside (IPTG) was added to a final concentration of 0.1 mM. After 3 h of additional incubation, the cells were collected by centrifugation, resuspended in 4 cm^3 of binding buffer (5 mM imidazole, 0.5 M NaCl, and 20 mM Tris-HCl, pH 7.9), and sonicated three times for 30 s, and the resultant suspension was then centrifuged for 30 min at 14 000 *g*. The supernatant was then applied to a 0.5 cm^3 of *His-Bind* (*Novagen*), which was washed with 5 cm^3 of binding buffer and 5 cm^3 of wash buffer (60 mM imidazole, 0.5 M NaCl, and 20 mM Tris-HCl, pH 7.9), then eluted with 3 cm^3 of 100 mM imidazole buffer (100 mM imidazole, 0.5 M NaCl, and 20 mM Tris-HCl, pH 7.9) followed by 3 cm^3 of 1 M imidazole buffer (1 M imidazole, 0.5 M NaCl, and 20 mM Tris-HCl, pH 7.9). The eluate generated in this step was then assessed for the presence of PgFPS by 10 % SDS-PAGE. The pellet of an induced culture of pLysS cells was resuspended in a 0.1 cm^3 of 50 mM Tris-HCl, pH 6.8, 10 mM DTT, 2 % SDS, 0.01 % bromophenol blue and 10 % glycerol. The 0.015 cm^3 aliquots were separated on 10 % gradient Tris-HCl polyacrylamide gels and stained with Coomassie blue for purposes of protein detection.

FPS activity assays were conducted in accordance with the methods described by Brodelius *et al*. (2002). Protein concentrations were determined by the Bradford method (Bradford 1976). Aliquots of bacterial extracts were assayed in 50 mM Tris-HCl, pH 8.0, containing 15 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 20 % glycerol, 55 μM GPP, and 50 μM [1-^{14}C] IPP, in a total volume of 0.05 cm^3. The reactions were incubated for 10 min at 30 °C, 6 M HCl was added to stop the reaction, and incubation was continued for a further 10 min in order to hydrolyze the FPP formed into extractable farnesol. Neutralization was performed with 6 M NaOH. The mixture was extracted with hexane, and the hexane phase was subsequently extracted with water. An aliquot of the extracted hexane phase was then counted using a scintillation counter. In order to analyze the radioactive reaction products, enzymatic reactions were conducted in accordance with the method described by Mekkriengkrai *et al*. (2004). The prenyl diphosphate products were extracted with 1-butanol saturated with water and then treated with potato acid phosphatase at 37 ºC overnight to hydrolyze the diphosphate moiety. The products were analyzed by reverse phase TLC (*LKC-18*, *Whatman*, Maidstone, UK) developed in acetone:water (9:1, v/v). The radioactive products were then identified by comparison with an authentic standard visualized by iodine vapour, and each of the radioactive spots was analyzed with a *Bio-image Analyzer BAS1500* (*Fuji*, Tokyo, Japan).

Southern blot analysis: Genomic DNA was isolated from the leaves of plants grown in field using the DNeasy plant mini kit (*Qiagen*). DNA was digested with *Eco*RI or *Sac*I, electrophoresed on 0.8 % agarose gel, and then transferred to a positively-charged nylon membrane (*Boehringer*, Mannheim, Germany) by capillary blotting (Sambrook *et al*. 1989). For Southern blot analysis, the full-length cDNA of *PgFPS* gene was labeled with the non-radioactive *AlkPhos* direct system (*Amersham*, Buckinghamshire, UK). Hybridization was for 12 h at 55 ºC and stringency washes were performed at 55 ºC. Hybridization signals were detected using *CDP-Star* detection kit (*Amersham*) according to the manufacturer's instructions.

Northern blot and RT-PCR analysis: 15 μg of ginseng total RNA from each sample was fractionated by electrophoresis in 1.5 % agarose gels containing 2.2 M formaldehyde. For the hybridization, full-length PCR-amplified *PgSS* (*P. ginseng* squalene synthase, AB115496) and *PgFPS* gene products labeled using digoxigenin (DIG)-dUTP (PCR DIG Probe Synthesis kit (*Roche*, Indianapolis, USA) were used as probes, respectively. DIG-labeled nucleic acid was hybridized and detected using the DIG Easy Hyb and DIG Nucleic Acid Detection kits (*Roche*). DNA was cross-linked to positively-charged nylon membranes and the membranes were incubated for 12 h at 48 °C with the probe, then washed twice at 25 °C, for 5 min each wash, in 2× SSC, 0.1 % SDS, followed by two washes, for 15 min each, at 68 °C in 0.5× SSC, 0.1 % SDS.

PgFPS gene expression in the different tissues was detected from the transcripts by RT-PCR. Total RNAs were prepared from the leaves, stems, roots, and seeds. RT-PCR analyses were conducted using the *AccessQuick RT-PCR System* (*Promega*). RNA (1 μg) was utilized as a template. For normalization, actin gene was employed as an external standard, as described by Gorpenchenko *et al*. (2006). RT-PCR conditions for both actin gene and *PgFPS* were as follows: one cycle at 45 °C for 45 min, and then 30 cycles at 95 °C for 1 min, 56 °C for 1 min, 72 °C for 1 min; and a final 5-min extension at 72 °C. The sizes of the PCR products were determined by agarose gel (1.5 %) electrophoresis.

Assay of FPS activity in hairy roots: Hairy roots (1 g) were ground in liquid nitrogen and resuspended in 1 cm^3 of Tris buffer (50 mM, pH 7.0), 5 mM DTT, 1 % polyvinylpolypyrrolidone (PVP-40), and 40 mM ascorbic acid, as described by Daudonnet *et al*. (1997). The mixture was filtered through two layers of *Miracloth* (*CalBiochem*, La Jolla, USA) and centrifuged at 17 700 *g*. The reaction was performed using 30 - 80 μg of protein per sample, as described by Masferrer *et al*. (2002).

Results and discussion

Cloning of a cDNA encoding FPS: To amplify the core fragments, we utilized degenerate primers designed from highly conserved regions of FPS (Pan *et al*. 1996). The forward primer was based on the consensus sequence CIEWLQAY, and the reverse primer was based on the consensus sequence QDDYLDCF. From the initial RT/PCR reaction, PCR products (520 bp) corresponding to the core fragment were cloned and sequenced. Based on this sequence, two RACE methods were conducted using sequence-specific primers in order to acquire the sequence information of the full-length cDNA encoding for FPS. Finally, a full-length cDNA encoding for FPS was isolated from the *P. ginseng* roots. The cDNA contained 1 344 bp, with an open reading frame (ORF) of 1 029 bp, flanked by a 109-bp 5'-untranslated region and a 205-bp 3'-untranslated region, including a 22 bp poly(A) tail. Two polyadenylation signals were identified either at 1 291 and or at 1 295 bp (AATAAA). This gene was deduced to code for a 343-amino acid protein with a calculated molecular mass of 39.6 kDa (data not shown). A *P. ginseng* gene encoding for FPS, designated *PgFPS*, has been submitted to the GenBank database (accession number DQ087959). The comparison of the deduced amino acid sequences of PgFPS with other plant FPSs shows high homologies in all conserved regions: 77, 84, 87 and 95 % identity with FPS from *Arabidopsis thaliana* (S52009), *Hevea barsiliensis* (S71454), *Artemisia annua* (P49350), and *Centella asiatica* (AAV58896), respectively. On the other hand, the protein showed low homology to FPS from other species: 23, 46, 47 and 52 % from *Escherichia coli* (BAA00599), *Homo sapiens* (NP001995), *Rattus norvegicus* (P05369), and *Saccharomyces cerevisiae* (P08524), respectively. These findings indicate that PgFPS is closely related to plant enzymes, and it differs significantly from mammalian and yeast enzymes.

Southern blot analysis: In order to estimate the copy number of the FPS gene in *P. ginseng*, Southern blotting was conducted using the full-length cDNA of *PgFPS* as a probe (Fig. 1). More than three bands were detected in the *Eco*RI and *Sac*I lane. These results suggest that the FPS gene might be a member of a small gene family. A small FPS gene family per genome has been reported in other plant species, including *Arabidopsis thaliana* (Cunillera *et al*. 1996) and *Ginkgo biloba* (Wang *et al*. 2004).

Characterization of *PgFPS* using recombinant protein: In order to characterize our *PgFPS* cDNA further, an expression plasmid was constructed via PCR to place the open reading frame ORF of *PgFPS* into the *Sac*I and *Xho*I sites of pET32a, yielding pET32a-*PgFPS*. In this vector *PgFPS* is fused to a C-terminal His-tag *via* 8 amino acids, and is fused to an N-terminal Trx/His/S-tag *via* 159 amino acids. When subjected to SDS-PAGE analysis (Fig. 2*A*), extracts of bacterial cells transformed with pET32a-*PgFPS* were determined to harbor the induced fusion protein (*lane 2*). The enzyme was then purified on a Ni^{2+} affinity column (*lane 3*). We employed the purified PgFPS enzyme for further detailed analyses. FPS catalyzes the consecutive condensations of DMAPP

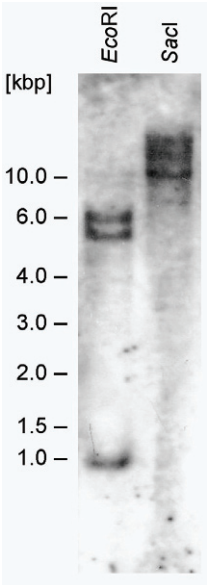

Fig. 1. Southern blot analysis of *PgFPS*. *Panax ginseng* genomic DNA was digested with different restriction endonuclease enzymes, separated by gel electrophoresis, and hybridized with the alkaline phosphate-labeled PCR product of overall ORF.

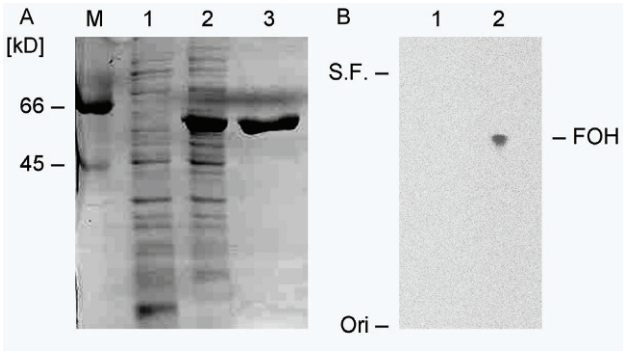

Fig. 2. SDS-PAGE analysis and identification of radioactive products. *A*: SDS-PAGE analysis of PgFPS expressed in *E. coli; lane* M - molecular mass markers, *lane* 1 - empty pET32a vector, *lane* 2 - pET32a containing ginseng *FPS*, *lane* 3 - $Ni2^{+}$ affinity purified PgFPS. The position of the PgFPS fused to Trx/His/S-taq at the N terminus and His-taq at the C terminus is approximately 56 kDa. *B*: Autoradiograph of the reverse-phase TLC of purified PgFPS reaction products. Reactions were incubated with [^{14}C]IPP in the presence and absence of GPP; *lane* 1 - the product was isolated from the reaction in which it was incubated in [^{14}C]IPP in the absence of GPP, *lane* 2, - the product was separated from the reaction in which it was incubated in [^{14}C]IPP in the presence of GPP; S.F. - solvent front, Ori - origin, FOH - farnesol.

or GPP with IPP to produce FPP as the final product. In this study, GPP was utilized as an allylic substrate in order to determine whether or not farnesol was generated from the reaction mixture. FPP should be converted into farnesol by hydrolysis to be isolated from the reaction mixture as the evidence of FPP formation. The reaction products were then dephosphorylated and analyzed by reverse-phase TLC (Fig 2*B*). In *lane 2*, farnesol (FOH) was detected, showing that PgFPS yielded FPP, whereas no farnesol was detected in *lane 1*. In order to compare the bacterial extracts induced from empty vector with pET32a-*PgFPS*, we conducted assays of FPS activities as described in the Materials and methods section. Extracts of cells expressing pET32a-*PgFPS* evidenced approximately 10-fold higher FPS activity compared to the extracts prepared from cells transformed with the empty vector [11 ± 0.02 and 1.20 ± 0.10 nmol(IPP) mg^{-1}(protein) min^{-1} in pET32a-*PgFSP* and pET32a (no insert), respectively]. These data confirm that the cDNA encodes FPS.

Expression analyses of *PgFPS* mRNA: In order to determine the pattern of *PgFPS* expression in different tissues, we conducted semi-quantitative RT-PCR (Fig. 3*A*). *PgFPS* transcripts could be detected in all tissues, but at different levels. The *PgFPS* transcript was detected in abundance in the roots and seeds, whereas it existed only at low levels in the leaves and stem. In particular, transcripts were detected at their highest levels in the roots; it is likely that sesquiterpene and triterpene compounds may be produced abundantly in the roots. This result is similar to that of the different patterns of *A. thaliana* FPS1S expression observed in different plant tissues (Cunillera *et al*. 1997). In *Arabidopsis*, the *FPS1* gene encoded for two isoforms, FPS1S and FPS1L. It has been reported that the function of FPS1S may function in a housekeeping capacity, to provide FPP for conversion into isoprenoids carrying out general cell functions.

Hairy roots were induced on ginseng, as the ability of hairy roots to grow to high density and produce significant quantities of secondary metabolites makes them a suitable system for the study of secondary metabolites. Elicitation with MJ has been shown to stimulate the induction of isoprenoid compounds in several plant cultures. Recently, these studies have also provided opportunities for preliminary metabolite profiling, gene discovery, and transcript expression analysis related to the triterpene pathway (Choi *et al*. 2005, Suzuki *et al*. 2002, 2005). However, more exact information regarding the *FPS* transcripts in plants treated with MJ was clearly necessary. We applied MJ to the ginseng hairy root cultures in order to gain greater insight into the functions of FPS in the hairy roots. mRNA levels and enzyme activities were monitored over a 7-d period, during which ginseng hairy root cultures were subjected to MJ treatment after 5 weeks of pre-cultivation. In Northern blot hybridization, *PgFPS* mRNA was readily detectable within 12 h of MJ treatment, reached maximum levels by 24 h, and declined to near control levels after 3 d of treatment (Fig. 3*B*). Farag *et al*. (2005) have reported similar enhancements of *FPS* transcripts in maize as a result of MJ treatment. When an overall ORF of the *PgSS* (*P. ginseng* squalene synthase) gene was employed as a probe, the same expression pattern as the *PgFPS* gene was observed (Lee *et al*. 2004). High levels of FPS activity were observed from 12 h to 7 d of MJ treatment, as compared to the control (Fig. 4). On the basis of our results, it appears that a *PgFPS* cloned from ginseng represents a favourable

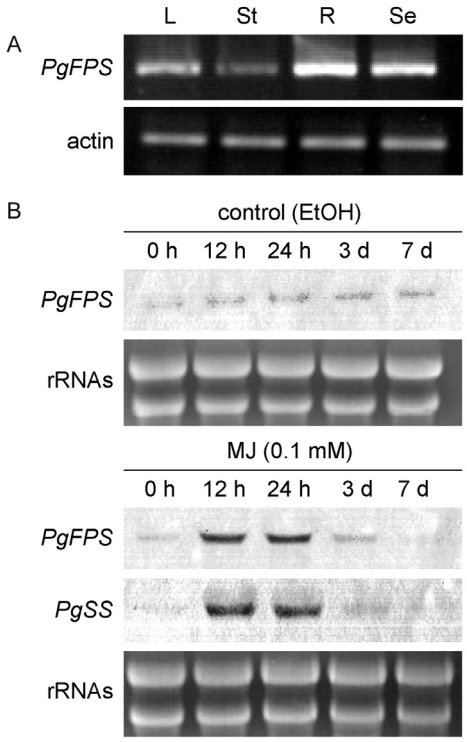

Fig. 3. *PgFPS* transcriptions in ginseng. *A*: *PgFPS* expression in different tissues of *P. ginseng*. Total RNA from leaf (L), stem (St), root (R), and seeds (Se) was utilized to determine the expression of *PgFPS* by RT-PCR. *B*: Time course of *PgFPS* and *PgSS* mRNA levels in the hairy roots of ginseng treated with 0.1 mM MJ and harvested at the times shown. Total RNA as template of RT-PCR was utilized for the normalization of RNA loading by actin gene or rRNAs.

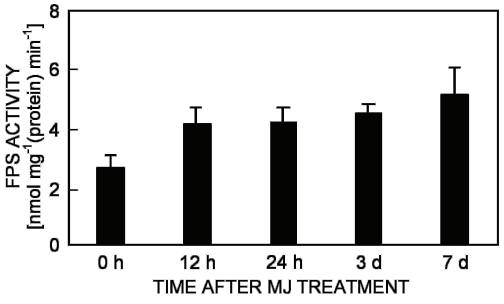

Fig. 4. PgFPS activity in *P. ginseng* hairy roots after treatment with 0.1 mM MJ. Means ± SE of three experiments.

candidate for studies of the effects of MJ on the carbon flux inherent to sesquiterpene and triterpene biosyntheses.

The results of several reports show that the gene transcriptions of squalene synthase, as well as squalene epoxidase and β-amyrin synthase associated with triterpene biosynthesis, are increased as a consequence of MJ treatment (Suzuki *et al*. 2002, Hayashi *et al*. 2003, Kim *et al*. 2005). Lee *et al*. (2004) demonstrated that *PgSS* transcription was observed at its highest levels in the adventitious roots of ginseng 24 h after MJ treatment. This gene performs a crucial role in the regulation of triterpene biosynthesis in roots overexpressing the *PgSS* gene. Lee *et al*. (2004) also noted that *PgSS* coupled with downstream genes is associated directly with triterpene biosynthesis, due to the increase in sterols and saponins caused by the overexpression of the gene. In the case of FPS, it has been reported that in transgenic tobacco (generated *via* the insertion of the heterologous *FPS* gene), both sterol and carotenoid biosynthesis are clearly increased as compared to controls (Daudonnet *et al*. 1997). Therefore, it can be concluded that FPS performs a crucial role in the regulation of the two biosynthetic pathways.

We cloned a full-length cDNA of *PgFPS*, which performs a role in the biosynthesis of isoprenoid compounds. There is, as yet, no evidence for the regulatory role of FPS in triterpene saponin biosynthesis, but our results raise the possibility that FPS may be a key regulatory enzyme in triterpene saponin biosynthesis, based on the data acquired in Northern blot analysis and FPS activity measurement, which revealed the upregulation of the *FPS* gene as the result of MJ treatment. In future studies, experiments involving the insertion of exogenous *FPS* genes into a plant will be required in order to determine whether or not FPS contributes to the synthesis of triterpene saponins. These experiments should entail the following: *1*) the generation of transgenic plants that overexpress *FPS*, and *2*) an investigation into the incorporation of radioactivity from FPP into triterpene saponins in transgenic plants. The results of these experiments will clarify the contribution of FPS to triterpene biosynthesis.

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