Abstract

Panax sokpayensis Shiva K. Sharma & Pandit is a new species of *Panax* reported from Sikkim Himalaya, India. Due to high medicinal value, its rhizomes are used in various preparations by local traditional healers for domestic and commercial use. Species of *Panax* are known to contain ginsenosides, which are the main bioactive principles responsible for their medicinal properties. Ginsenosides are triterpene glycoside saponins with cardioprotective, immunomodulatory, antifatigue, anticancerous, antidiabetic and antioxidant properties. They are synthesized through ginsenoside biosynthetic pathway which derives its precursors from mevalonate (MVA) and non-mevalonate (MEP) pathways as reported in different *Panax* species. In the current work, several genes of pathways involved in ginsenoside biosynthesis were cloned and characterized. As there was no scientific validation of the ginsenosides content of *P. sokpayensis*, major ginsenosides' profile of this important medicinal plant was also studied.

Suppression subtractive hybridization (SSH) studies generated 513 and 374 high quality Expressed Sequence Tags (ESTs) in the leaf and rhizome libraries, respectively. The ESTs of leaf library assembled into 80 unigenes while that of rhizome assembled into 160 unigenes. Three ginsenoside biosynthetic pathway genes, viz., *farnesyl pyrophosphate synthase* (*PsFPS*), *squalene synthase* (*PsSS*) and *dammarenediol synthase* (*PsDS*) were detected in the rhizome SSH library. Moreover, 13.75 % of unigenes from the leaf SSH library were not represented in the available leaf transcriptome of *P. ginseng* and around 18.12, 23.75, 25, and 6.25 % of unigenes from the rhizome SSH library were not represented in the available root/rhizome transcriptomes of *P. ginseng*, *P. notoginseng*, *P. quinquefolius* and *P. vietnamensis*, respectively. These rare and novel unigenes could complement the available *Panax* transcriptomes and could be valuable resources for gene discovery in *P. sokpayensis*.

Seventeen partial fragments belonging to MVA, MEP and ginsenoside biosynthetic pathways were cloned using degenerate primer approach. These partial fragments were of the following genes: a*cetyl-CoA C-acetyltransferase* (*PsAACT*), *3-hydroxy-3-methylglutaryl coenzyme A synthase* (*PsHMGS*), m*evalonate kinase* (*PsMVK*), *phosphomevalonate kinase* (*PsPMVK*), *1-deoxy-D-xylulose-5-phosphate synthase* (*PsDXS*), *1-deoxy-D-xylulose 5-phosphate reductoisomerase* (*PsDXR*), *2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase* (*PsCMS*), *4-*(*cytidine 5'-diphospho*)*-2-C-methyl-D-erythritol kinase* (*PsCMK*), *2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase* (*PsMCS*), *4-hydroxy-3-*

methylbut-2-en-1-yl diphosphate synthase (*PsHDS*), *4-hydroxy-3-methylbut-2-enyl diphosphate reductase* (*PsHDR*), *isopentenyl diphosphate isomerase 2* (*PsIDI2*), *squalene epoxidase* (*PsSE*), *β-amyrin synthase* (*Psβ-AS*), *protopanaxadiol synthase* (*PPDS*), *protopanaxatriol synthase* (*PPTS*) and *cycloartenol synthase* (*PsCS*).

Full length cDNAs of seven genes related to ginsenoside biosynthesis, viz., *PsFPS*, *PsSS*, *PsDS*, *PsPMVK*, *PsCMK*, *PsSE* and *PsCS* were successfully cloned through rapid amplification of cDNA ends (RACE). *In silico* characterizations of the deduced polypeptides of these genes detected residues and domains that are responsible for the activities of the respective proteins. These were highly conserved across different *Panax* species and across different genera and families. The quantitative realtime PCR (qRT-PCR) studies on *PsFPS*, *PsSS*, *PsDS*, *PsSE* and *PsCS* revealed that these genes were differentially expressed among leaf, stem and rhizome tissues indicating their possible roles in regulation of ginsenoside biosynthesis.

Using ORF of *PsFPS* and pQE30 expression vector, recombinant PsFPS was successfully produced in *E. coli*. The time course analysis of expression of recombinant protein revealed high level of expression within 1 h of induction by 1 mM IPTG when the curde extracts were analyzed on 12 % SDS-PAGE.

Using High Herformance Liquid Chromatography (HPLC), all the major ginsenosides, viz., Rb1, Rb2, Rc, Rd, Re, Rf, Rg1 and Rg2 that were tested in the rhizome of 10 years old *P. sokpayensis*, were detected and quantified except for Rc. The total ginsenoside content (28.53 ± 2.07 mg/g of dry weight) was found to be at par with the ginsenoside content of its Asian congener. Also the Rb1:Rg1 ratio of 2.13 is characteristic of an Asian *Panax* spp.

Seven full length and thirteen partial sequences of genes belonging to MVA, MEP and ginsenoside biosynthetic pathways cloned in the present work would be useful in further characterizations of these genes, including the regulatory roles played by them. Also, the successful heterologous production of PsFPS in *E. coli* could pave the way for the similar production of other enzymes of the pathway using the genes cloned above which would lead to an alternative approach to synthesize ginsenosides or their intermediates through synthetic biology. Moreover, our work for the first time scientifically validated the presence of ginsenosides in *P. sokpayensis* which might serve as a base for further phytochemical studies as well as commercialization of this important medicinal plant.