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Expression profiling of the triterpene saponin biosynthesis genes *FPS*, *SS*, *SE*, and *DS* in the medicinal plant *Panax notoginseng*

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ABSTRACT

Panax notoginseng (Burk) F. H. Chen, an economically significant medicinal plant with hemostatic and health tonic activities, has been used in Traditional Chinese Medicine (TCM) for more than 3000 years. Triterpene saponins are responsible for most of the pharmacological activities of *P. notoginseng*. Here, we cloned five cDNA sequences encoding the key enzymes involved in triterpene saponin biosynthesis, namely, *PnFPS*, *PnSS*, *PnSE1*, *PnSE2*, and *PnDS*, and analyzed the conserved domains and phylogenetics of their corresponding proteins. Their organ-specific expression patterns in four-year-old *P. notoginseng* were detected by real-time PCR, showing that they were all most highly expressed in flowers. In addition, four of the genes, excluding *PnSE2*, were upregulated in leaves following stimulation with methyl jasmonate. This study is the first comprehensive analysis of the expression patterns of pivotal genes for triterpene saponin biosynthesis in *P. notoginseng* and provides a basis to further elucidate the molecular mechanism for the biosynthesis of these medically important compounds.

1. Introduction

Panax notoginseng (Burk) F. H. Chen, also called Sanchi, has been cultivated and used in medicine for thousands of years in China for its remarkable and valuable hemostatic effect. The herb possesses antihypertensive, anti-thrombotic, anti-atherosclerotic, hepatoprotective, and neuroprotective activities (Ng, 2006) and is a major component of common household medicines (Liu et al., 2008; Zhang and Cheng, 2006) such as the Yunnan Paiyao powder and the compound Danshen dripping pill. The herb has multiple active constituents (Dan et al., 2008; Gao et al., 1996; Wei et al., 1980), including triterpene saponins, phytosterols, flavonoids, and polysaccharides. The major bioactive components are triterpene saponins. Saponins possess a triterpene skeleton (aglycone) derived from the precursor oxidosqualene (C30) to which glycosyl residues are attached (Vincken et al., 2007). Approximately 60 different triterpene saponins have been isolated and characterized (*e.g.* ginsenosides, notoginsenosides, and gypenosides) (Wang et al., 2006; Wei et al., 1992). According to the structures of the aglycones, all saponins are dammarane-type glycosides (Wang et al., 2006). The saponins have been classified into two groups: protopanaxadiols (*e.g.* Rb1, Rd) and protopanaxatriols (*e.g.* Rg1, Rg2). The oleanane-type saponin Ro, which exists in other major *Panax* plants, including *P. ginseng* and *P. quinquefolius*, has not been isolated from *P. notoginseng* (Wang et al., 2006). Each of the saponins is reported to exhibit different bioactive effects based on previous pharmacological studies (Sun et al., 2005). However, the development of *P. notoginseng* for pharmaceutical uses has faced various challenges, such as difficulties in long-term conventional cultivation, obstacles to continuous cropping, and a shortage of cultivated lands (Jia et al., 2013; Ou et al., 2012). To ensure the continued development of this herb, comprehensive and in-depth studies on the synthesis of triterpene saponins in *P. notoginseng* at the molecular level must be performed both *in vivo* and *in vitro*.

Triterpene saponins are biosynthesized through the mevalonic acid (MVA) pathway (Fig. 1), which involves the sequential conversion of farnesyl diphosphate (FPP) to squalene and then to 2,3-oxidosqualene, followed by a series of cyclization, oxidation, hydroxylation, and glycosylation reactions (Augustin et al., 2011). Farnesyl diphosphate

Abbreviations: MeJA, methyl jasmonate; Ct, cycle threshold; ORF, open reading frame; EST, expressed sequence tag; NCBI, National Center for Biotechnology Information; kDa, kilo Dalton; pI, isoelectric point; MVA, mevalonic acid; FPP, farnesyl diphosphate; FPS, farnesyl diphosphate synthase; SS, squalene synthase; SE, squalene epoxidase; DS, dammarenediol-II synthase; OSCs, oxidosqualene cyclases; CYP450, cytochrome P450-dependent monooxygenases; GT, glycosyltransferases; NJ, neighbor-joining; ML, maximum likelihood; R, root; S, stem; L, leaf; F, flower.

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synthase (FPS, EC 2.5.1.1) catalyzes the sequential condensations of dimethylallyl diphosphate and geranyl diphosphate with isopentenyl diphosphate to produce FPP, thereby providing substrate for the synthesis of phytosterols, sesquiterpenes, and triterpenes (Cunillera et al., 1996). Squalene synthase (SS, EC 2.5.1.21) converts two FPP molecules into a C30 isoprenoid squalene, which is an essential substrate for the biosynthesis of cholesterol, steroid hormones, vitamin D, and triterpene (Jarstfer et al., 2002). The first oxygenation step in phytosterol and triterpenoid biosynthesis is performed by squalene epoxidase (SE, EC 1.14.99.7), which catalyzes the epoxidation of the double bond of squalene to form 2, 3-oxidosqualene (Laden et al., 2000). Dammarenediol-II synthase (DS) belongs to the oxidosqualene cyclase (OSC, EC 6.5.99.7) family and catalyzes the cyclization of 2, 3-oxidosqualene to form various secondary metabolites (Fig. 1). Further diverse oxidation, hydroxylation, and glycosylation modifications are catalyzed by cytochrome P450-dependent monooxygenases (CYP450) and glycosyltransferases (GT), finally producing multiple ginsenosides with a variety of structures and biological activities.

Several genes associated with triterpenoid biosynthesis have been cloned and characterized in many different species, and reports have indicated that changes in gene expression could enhance the production of secondary metabolites (Han et al., 2012; He et al., 2008; Kim et al., 2011). Chen et al. (2000) demonstrated that the overexpression of the *FPS* gene could upregulate sesquiterpene biosynthesis in *Artemisia annua* L. The overproduction of triterpene saponins in cell suspension or adventitious root cultures was successfully induced by the methyl jasmonate (MeJA) elicitor in both *P. ginseng* and *P. notoginseng* (Hu and Zhong, 2007; Kim et al., 2009). Luo et al.

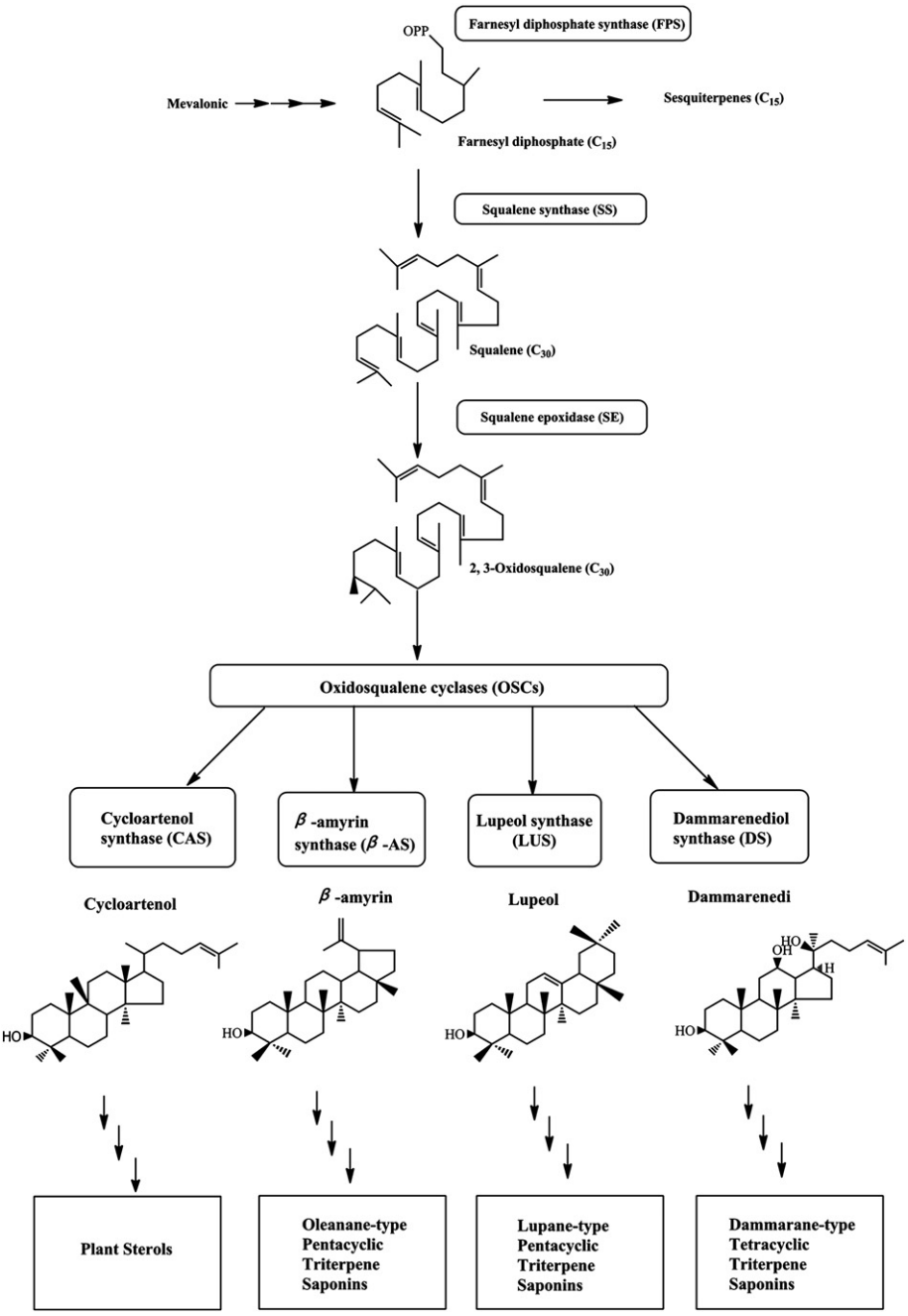

Fig. 1. Putative pathway for triterpene biosynthesis in plants.