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Research article

Alteration of *Panax ginseng* saponin composition by overexpression and RNA interference of the protopanaxadiol 6-hydroxylase gene (*CYP716A53v2*)

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ABSTRACT

Background: The roots of *Panax ginseng* contain noble tetracyclic triterpenoid saponins derived from dammarenediol-II. Dammarene-type ginsenosides are classified into the protopanaxadiol (PPD) and protopanaxatriol (PPT) groups based on their triterpene aglycone structures. Two cytochrome P450 (CYP) genes (*CYP716A47* and *CYP716A53v2*) are critical for the production of PPD and PPT aglycones, respectively. CYP716A53v2 is a protopanaxadiol 6-hydroxylase that catalyzes PPT production from PPD in *P. ginseng*.

Methods: We constructed transgenic *P. ginseng* lines overexpressing or silencing (via RNA interference) the *CYP716A53v2* gene and analyzed changes in their ginsenoside profiles.

Result: Overexpression of *CYP716A53v2* led to increased accumulation of *CYP716A53v2* mRNA in all transgenic roots compared to nontransgenic roots. Conversely, silencing of *CYP716A53v2* mRNA in RNAi transgenic roots resulted in reduced *CYP716A53v2* transcription. *HPLC* analysis revealed that transgenic roots overexpressing *CYP716A53v2* contained higher levels of PPT-group ginsenosides (Rg_1, Re, and Rf) but lower levels of PPD-group ginsenosides (Rb1, Rc, Rb_2, and Rd). By contrast, RNAi transgenic roots contained lower levels of PPT-group compounds and higher levels of PPD-group compounds.

Conclusion: The production of PPD- and PPT-group ginsenosides can be altered by changing the expression of *CYP716A53v2* in transgenic *P. ginseng*. The biological activities of PPD-group ginsenosides are known to differ from those of the PPT group. Thus, increasing or decreasing the levels of PPT-group ginsenosides in transgenic *P. ginseng* may yield new medicinal uses for transgenic *P. ginseng*.

1. Introduction

Triterpenoid saponins are present in many higher plants and exhibit a wide range of biological activities depending on their structures. Certain triterpenoid saponins also have commercial value and are exploited as drugs and medicines [1–3], and saponins may have natural roles in the defense against pathogens and pests [4].

The roots of ginseng (*Panax ginseng* Meyer) contain pharmacologically active components. It is generally believed that ginsenoside saponins are the main compounds responsible for the pharmacological activities of *P. ginseng* [2,3]. Ginsenosides are classified as protopanaxadiol (PPD) or protopanaxatriol (PPT) saponins based on their aglycone structures. PPD-group ginsenosides have glycosidic bonds at the C-3 and C-20 hydroxyl groups, and the major ginsenosides of this group include Ra1, Ra2, Rb1, Rc, Rd, and Rg3. PPT-group ginsenosides have glycosidic bonds at the C-6 and C-20 hydroxyl groups, and the major ginsenosides of this group include Re, Rf, Rg1, Rg2, and Rh1.

The first step in ginsenoside biosynthesis is the cyclization of 2,3-oxidosqualene to dammarenediol-II, which is catalyzed by dammarenediol synthase of the oxidosqualene cyclase group [5].

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Dammarenediol-II is hydroxylated by two cytochrome P450 enzymes (CYP716A47 and CYP71653v2) to produce PPDs and PPTs [6,7]. These compounds are subsequently glycosylated by glycosyltransferase (Fig. 1). The CYP716A47 enzyme in *P. ginseng* catalyzes the hydroxylation of dammarenediol-II at the C-12 position to yield PPD, which is further hydroxylated at its C-6 position by CYP71653v2 to yield PPT.

PPD-group ginsenosides have biological activities that are opposite of those in the PPT group [8,9]. For example, Rg1 (PPT group) stimulates the central nervous system, whereas Rb1 (PPD group) suppresses the activity of the central nervous system [8,9].

Because the two CYP genes (*CYP716A47* and *CYP71653v2*) are necessary for producing PPD and PPT aglycones, respectively, we postulated that modifying the expression of these genes in *P. ginseng* could alter the composition of ginsenosides. Transgenic ginseng with altered ginsenoside profiles could be used for new medicinal applications requiring precise tuning of pharmacological activity. In this study, we constructed two transgenic *P. ginseng* lines, with overexpression or silencing [RNA interference (RNAi)] of *CYP716A53v2*, and we analyzed the changes in their PPD and PPT ginsenoside levels. We found that the production of PPT-group ginsenosides was altered by both the overexpression and RNAi-based silencing of the *CYP716A53v2* gene.

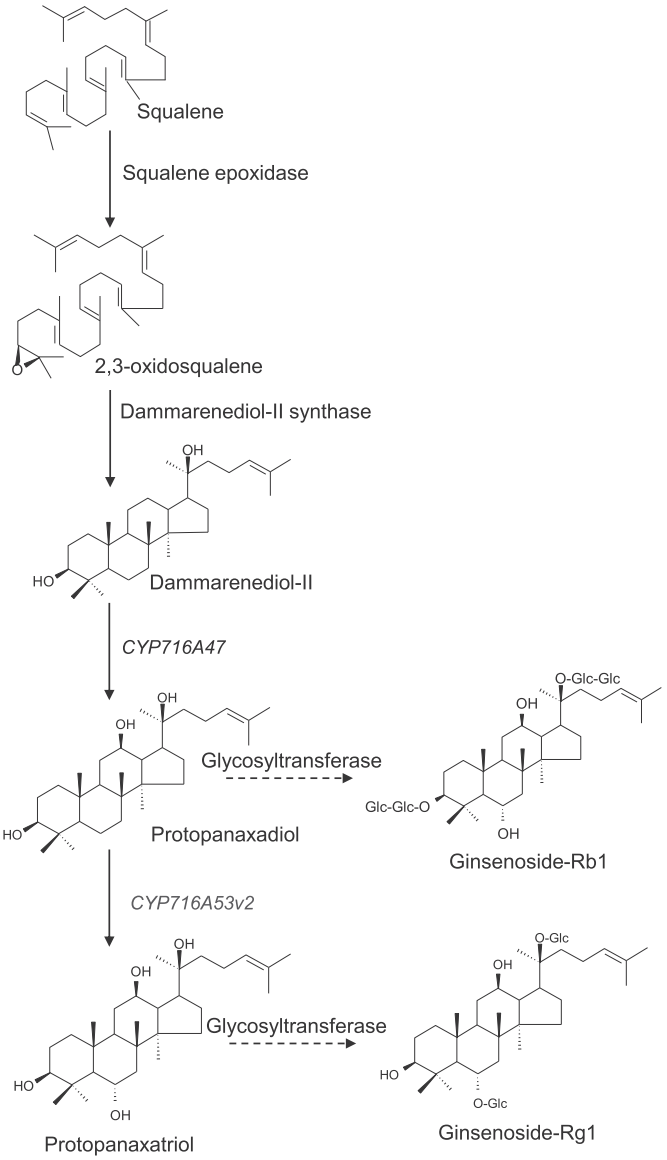

Fig. 1. Biosynthetic pathway for ginsenosides in *Panax ginseng*. Squalene epoxidase converts squalene to 2,3-oxidosqualene, which is then converted to a triterpene aglycone (dammarenediol-II) by dammarenediol synthase. Dammarenediol-II undergoes oxidation and glycosylation and is finally converted to protopanaxadiol (PPD)- and protopanaxatriol (PPT)-group ginsenosides.

2. Materials and methods

2.1. Overexpression and RNAi silencing vector construction

The ORF (open reading frame) region of the *CYP716A53v2* sequence was cloned into the pCR 8.0 vector (Invitrogen Life Technologies, Carlsbad, CA, USA) and then transferred to the destination vector pH2WG to yield the *CYP716A53v2* overexpression vector. To construct the *CYP716A53v2*-RNAi vector, two primers including gateway adapters (Invitrogen Life Technologies) were designed to amplify the region from 1,259 bp to 1,498 bp in *CYP716A53v2*. The amplified polymerase chain reaction (PCR) product was cloned into the pSB1 vector and then transferred to the RNAi destination vector pB7GWIWG2(II) (which contains the *BAR* gene that confers Basta resistance to plant cells) in *Escherichia coli* DH5α, as described by the manufacturer (Invitrogen Life Technologies). The construct was sequenced and subsequently transformed into *Agrobacterium tumefaciens* GV3101 cells harboring plasmid pMP90 using standard molecular biology techniques.

2.2. Construction of transgenic P. ginseng

The generation of transgenic *P. ginseng* was conducted as described in our previous report [10]. Putative transgenic somatic embryos for both *CYP716A53v2* overexpression and *CYP716A53v2*-RNAi were transferred to the selection medium, with additional supplementation of 20μM GA_3, to induce embryo germination. The plantlets were maintained on 1/2-strength MS medium with 2% sucrose.

Selection of transgenic root lines was performed as described by Han et al [5]. As a nontransgenic control, adventitious roots were induced from the *in vitro* maintained nontransformed plants that were the original sources of the transgenic plants.

2.3. Reverse transcription-PCR in transgenic roots

Total RNA was isolated from nontransgenic and transgenic roots and reverse-transcribed using the ImProm-II Reverse Transcription System (Promega, Madison, WI, USA). First-strand cDNA was used as the template for the reverse transcription (RT)-PCR analysis, which was performed as follows: 96°C for 5 min; 30 cycles of 96°C for 30 s, 60°C for 30 s, 72°C for 1 min; and a final extension at 72°C for 10 min. *β-Actin* cDNA (primers 5′-ATG GTC AAG GCT GGA TTT GCA-3′ and 5′-CTC GAC CAG CTA AAT CAA GAC G-3′) was used as a control for RNA integrity and loading accuracy. The RT-PCR analyses were performed twice, and representative data are shown in the figures. The primers used for amplification were 5′-ATG GAT CTC TTT ATC TCA TCT CAA-3′ and 5′-TTA AAG CGT ACA AGG TGA TAG ACG-3′ for *P. ginseng CYP716A53v2*, 5′-GCG TGA CCT ATT GCA TCT CC-3′ and 5′-TTC TAC ACA GCC ATC GGT CC-3′ for the hygromycin phosphotransferase gene (*HPT*), and 5′-AGG ACA GAG CCA CAA ACA CC-3′ and 5′-ATG CTT GTA TCC AGC TGC G-3′ for the phosphinothricin acetyl transferase gene (*BAR*).