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ORIGINAL PAPER

# Functional analysis of β-amyrin synthase gene in ginsenoside biosynthesis by RNA interference

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**Abstract**

***Key message*** **Down-regulation of β-amyrin synthase gene expression by RNA interference led to reduced levels of β-amyrin and oleanane-type ginsenoside as well as up-regulation of dammarane-type ginsenoside level.**

*Abstract* In the biosynthetic pathway of ginsenosides, β-amyrin synthase catalyzes the reaction from oxidosqualene to β-amyrin, the proposed aglycone of oleanane-type saponins. Here, RNAi was employed to evaluate the role of this gene in ginsenoside biosynthesis of *Panax ginseng* hairy roots. The results showed that RNAi-mediated down-regulation of this gene led to reduced levels of β-amyrin and oleanane-type ginsenoside $R_o$ as well as increased level of total ginsenosides, indicating an important role of this gene in biosynthesis of ginsenoside. Expression of key genes involved in dammarane-type ginsenoside including genes of dammarenediol synthase and protopanaxadiol and protopanaxatriol synthases were up-regulated in RNAi lines. While expression of squalene synthase genes was not significantly changed, β-amyrin oxidase gene was down-regulated. This work will be helpful for further understanding ginsenoside biosynthesis pathway.

**Keywords** Ginsenoside · β-Amyrin synthase · RNAi · Hairy roots

**Abbreviations**

| | |
|---|---|
| βAS | β-Amyrin synthase |
| MeJA | Methyl jasmonate |
| RNAi | RNA interference |
| HPLC | High-performance liquid chromatography |

## Introduction

Ginsenosides are considered to predominantly account for the pharmacological activities of *Panax ginseng* including anti-cancer, anti-fatigue, and anti-diabetes effects (Leung and Wong 2010). Biosynthetic studies have suggested that ginsenosides should be biosynthesized from oxidosqualene (Fig. 1) (Christensen 2009; Liang and Zhao 2008). Oxidosqualene could be transformed to the precursors of ginsenosides including dammarenediol and β-amyrin, under the catalysis of dammarenediol synthase and β-amyrin synthase (βAS), respectively. Thus, ginsenosides could be classified into dammarane-type and oleanane-type saponins, which are derived from dammarenediol and β-amyrin, respectively. So far, more than 40 ginsenosides have been isolated from ginseng, among which only the ginsenoside $R_o$ was identified as oleanane-type.

Benefiting from the fast development of modern molecular biology technic, the biosynthetic pathway of ginsensoides is becoming more and more clear. Several genes involved in ginsenoside synthesis have been cloned and identified, including genes of farnesyl diphosphate synthase (Kim et al. 2010), squalene synthase (Lee et al. 2004), dammarenediol synthase (Tansakul et al. 2006), β-amyrin synthase (Kushiro et al. 1998), and Cyt P450 enzymes catalyzing the formation of protopanaxadiol from

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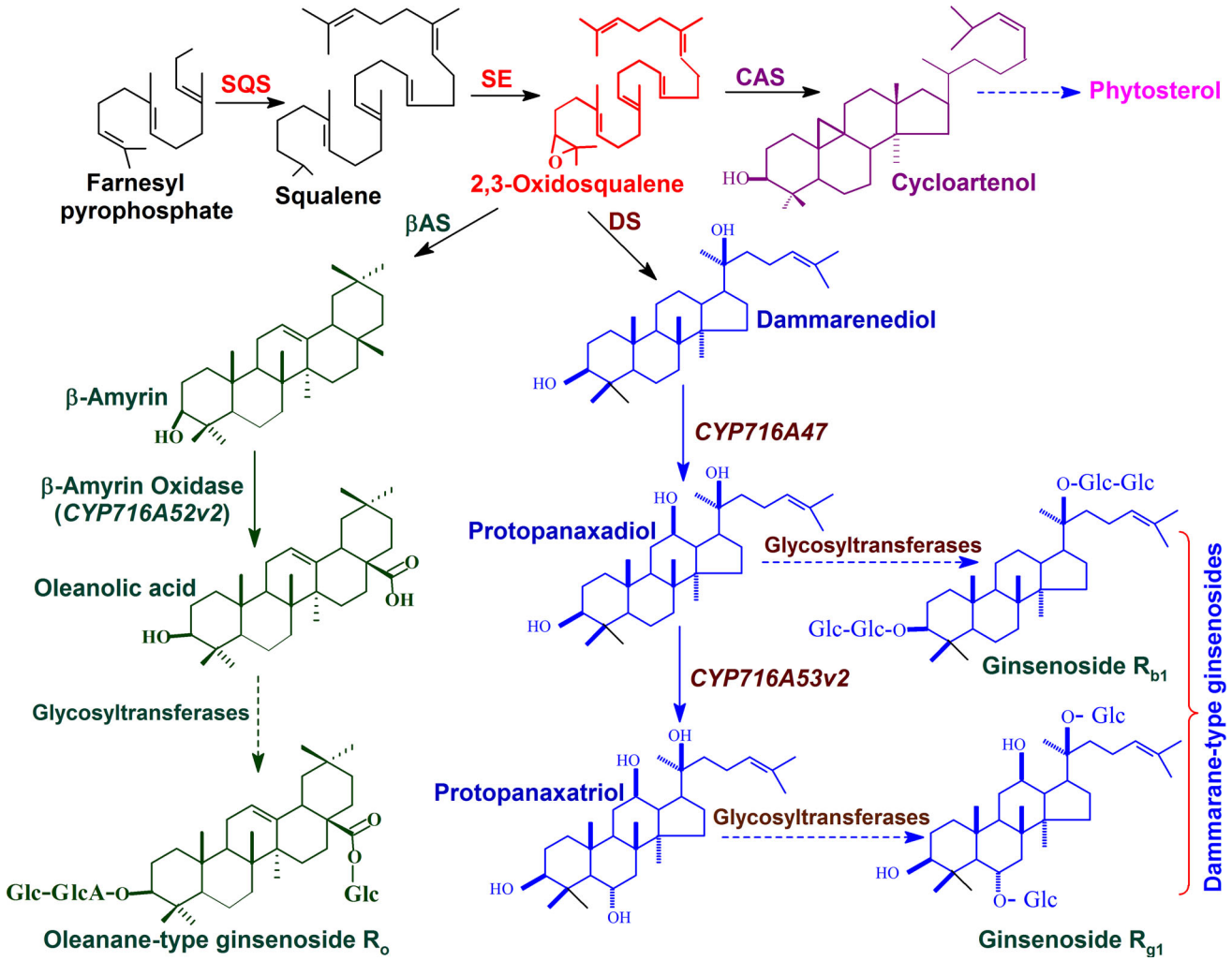


**Fig. 1** The proposed biosynthetic pathway for ginsenosides. Squalene formation from farnesyl pyrophosphate derived from mevalonate or non-mevalonate pathway is catalyzed by squalene synthase (SQS). Ginsenosides are derived from the same precursor, 2,3-oxidosqualene, which is synthesized from squalene under the catalysis of squalene epoxidase (SE). Oxidosqualene was further transformed to dammarenediol and β-amyrin, under the catalyzation of dammarenediol synthase and β-amyrin synthase, respectively. Furthermore, dammarenediol is transformed into dammarane-type ginsenosides, while β-amyrin is modified to oleanane-type ginsenosides. On the other hand, 2,3-oxidosqualene is also transformed to cycloartenol under the catalysis of cycloartenol synthase (CAS) for phytosterol synthesis

dammarenediol, protopanaxatriol from protopanaxadiol, and oleanolic acid from β-amyrin (Han et al. 2011, 2012, 2013). Moreover, many potential candidate genes involved in ginsenoside biosynthesis have been revealed by several previous *P. ginseng* transcriptome studies (Chen et al. 2011; Jung et al. 2003; Kim et al. 2006; Li et al. 2013; Sathiyamoorthy et al. 2010a, b). Indubitably, these data will continue to contribute to elucidation of ginsenoside anabolism in *P. ginseng*. Due to their low sequence conservatism, clone and identification of glycosyltransferases involved in ginsenoside biosynthesis represent a major obstacle in dissecting ginsenoside pathway. Nevertheless, progress has been made in this regard. Based on ginseng transcriptome data, two UDP-glycosyltransferases were identified, namely PgUGT74AE2 and PgUGT94Q2. PgUGT74AE2 transfers a glucose moiety from UDP-glucose (UDP-Glc) to the C3 hydroxyl groups of protopanaxadiol and compound K to form Rh2 and F2, respectively, whereas PgUGT94Q2 transfers a glucose moiety from UDP-Glc to Rh2 and F2 to form Rg3 and Rd, respectively (Jung et al. 2014). Additionally, a glucosyltransferase named as UGTPg1 was cloned and identified from *P. ginseng* recently. Heterologous expression of this enzyme in yeast indicated that it could catalyze the transformation from dammarenediol to 20*S*-*O*-β-(D-glucosyl)-dammarenediol, protopanaxadiol to compound K, ginsenoside Rg3 to Rd, and Rh2 to F2 (Yan et al. 2014). Undoubtedly, strategies used in these studies will provide

important enlightenment for cloning and identification of glycosyltransferases involved in ginsenoside or other secondary metabolite biosynthesis.

RNA interference (RNAi) is a quick, easy, and sequence-specific way to inhibit the expression of chosen genes. RNAi is now widely used in plant biotechnology, both as an useful tool for discovering or validating gene functions as well as a quick way of engineering specific reductions in expression of chosen genes (Small 2007; Watanabe 2011). Hairy roots induced by infection of plants with *Agrobacterium rhizogenes* were recommended as an excellent system for plant secondary metabolite production and relative research fields, due to their genetic stability, rapid growth, and easiness for maintenance (Banerjee et al. 2012; Ono and Tian 2011; Zhou et al. 2011). The *βAS* of *P. ginseng* has been cloned and identified (Kushiro et al. 1998), providing possibility for further functional analysis of this gene. Taking advantage of *P. ginseng* hairy root culture system, we employed RNAi in this study to provide some evidences for the involvement of *βAS* in ginsenoside biosynthesis.

## Materials and methods

### Establishment of RNAi component and RNAi hairy root lines

*βAS*-RNAi component was constructed by gateway strategy. Because there are two isoenzyme genes (*PNY1* and *PNY2*) encoding β-amyrin synthase in *P. ginseng*, we selected a domain that is highly conserved at nucleotide level within both genes to construct *βAS*-RNAi component. Based on the nucleotide sequence of *PNY1*, primers were designed as P1 (5′AAA AAG CAG GCT TGC CAA GGA GGA CAT CTA 3′) and P2 (5′AGA AAG CTG GG A TCC CAT TCC TGA CTA CC 3′) containing gateway adapters (Invitrogen Life Technologies, Carlsbad, CA, USA) indicated by underline. A *βAS*-specific fragment (334 bp, from 1037 to 1370) was amplified by RT-PCR with total RNA from *P. ginseng* hairy roots as template, isolated by RNAiso Reagent (TaKaRa Dalian Biotechnology Co., Ltd.) according to the manufacturer's protocol. Hairy roots of *P. ginseng* used for total RNA isolation were previously established in our laboratory and maintained by subculture on solid 1/2 MS medium at 25 °C and 4-week intervals (Liang et al. 2009). PCR product was inserted into pDONR$^{TM}$ vector and recombined into the RNAi destination vector pK7GWIWG2 (II). The expression of the recombined segment is driven by the cauliflower mosaic virus 35S promoter (P35S). The resulted RNAi vector was transformed into *Escherichia coli* DH5α, sequenced, and subsequently transformed into *A. rhizogenes* A4 using a standard procedure. Roots of 3-year-old *P. ginseng*, which were kindly contributed by Chinese Institute of Special Economic Animal and Plant Science, were used to induce *βAS*-RNAi hairy root lines with the positive A4 strains according to a method in our laboratory (Liang et al. 2009). Hairy roots transformed by *A. rhizogenes* A4 harboring empty vector were used as controls. The nature of the hairy roots was confirmed by sequencing the PCR-amplified rolC genes with primers P3 and P4 (5′-CGA AGC TTC TATA TTC GCA CAA CG-3′ and 5′-GCG AAT TCG CGA TGA AAT CAA GTA TCC-3′). RNAi lines were prescreened by hygromycin (15 mg/L) resistance and further confirmed by sequencing of the PCR products with hairy root DNA as template and attBF and attBR (5′-GGG GAC AAG TTT GTA CAA AAA AGC AGG CT-3′ and 5′-GGG GAC CAC TTT GTA CAA GAA AGC TGG G-3′) as primers.

### Elicitation by methyl jasmonate

Five hairy roots tips ~20 mm long (0.02 g) were inoculated in liquid 1/2 MS medium. The medium of 15-day-old hairy root culture was replaced by fresh medium of the same volume containing methyl jasmonate (MeJA, 0.1 mM).

### Quantitative PCR

Quantitative PCR was carried out to assess the effect of RNAi on expression of genes involved in ginsenoside biosynthesis. Total RNA was isolated from the hairy roots. Primers were designed as described in Table 1. PCR reaction was run using the Qiagen Quantitect SYBR Green PCR system according to the direction. A denaturation step at 95 °C for 15 min was run prior to the amplification in 3 steps: denaturation at 94 °C for 15 s, annealing at 55 °C for 15 s, and 20 s of extension at 72 °C, with a total of 40 cycles. The gene expression levels of treated roots relative to the untreated roots were calculated by the comparative $C_T$ method ($2^{-\Delta\Delta C_T}$) with *Actin* as an internal control (Schmittgen and Livak 2008).

### Chemical analysis

Analysis of total ginsenosides and dammarenediol was performed according to the protocol reported in our lab (Liang et al. 2009; Zhao et al. 2014). Ginsenosides $Rb_1$, $Rg_1$, and Ro were quantitatively analyzed by HPLC using a previously reported protocol in our lab (Zhao et al. 2014).

β-Amyrin was analyzed by high-performance liquid chromatography (HPLC). *P. ginseng* hairy root powder of 1 g within 10 ml methanol was sonicated at 40 kHz and 30 °C for 30 min. The extract was membrane-filtered,

**Table 1** Primers designed for qPCR

| Genes | Accession no. | Primers |
|---|---|---|
| β-Amyrin synthase (*PNY1*) | AB009030 | 5′-ATGTGGAAGCTTAAGATAGCGG-3′ |
| | | 5′-TTAGGTGCCTAGGGACGGTAAT-3′ |
| β-Amyrin synthase (*PNY2*) | AB014057 | 5′-ATTACCAGTAACAGAAGAACT-3′ |
| | | 5′-AGAGTAAATAAGTGCCCTAC-3′ |
| Squalene synthase (*PgSS1*) | AB115496 | 5′-ATG GGAGTTTGGGGGCAATTCT-3′ |
| | | 5′-GTTCTCACTGTTTGTTCAGTAGTAGGTT-3′ |
| β-Amyrin oxidase (*CYP716A52v2*) | JX036032 | 5′-ATGTCCCTCTCCTCTCACTCTTTG-3′ |
| | | 5′-TTAGGCTTTGTGTGGAAATAGGCG-3′ |
| Protopanaxatriol synthase (*CYP716A53V2*) | JX036031 | 5′-ATGGATCTCTTTATCTCATCTCAA-3′ |
| | | 5′-TTAAAGCGTACAAGGTGATAGACG-3′ |
| Protopanaxadiol synthase (*CYP716A47*) | JN604537 | 5′-ATG GTGTTGTTTTTCTCCCTATCT-3′ |
| | | 5′-TTAATTGTGGGGATGTAGATGAAT-3′ |
| Dammarenediol synthase (*PNA*) | AB265170 | 5′-ATGTGGAAGCTGAAGGTT GCTCAAGGA-3′ |
| | | 5′-TTAAAT TTTGAGCTGCTGGTGCTTAGGC-3′ |
| Cycloartenol synthase (*CAS*) | AB009029 | 5′-TTCCCAGACCTTGTCCAGTGATGCCCAAAG-3′ |
| | | 5′-GCCTGAAGAGCTTGCGGAGGTCGAGAAAGT-3′ |

*CYP716A47* dammarenediol 12-hydroxylase, *CYP716A53V2* protopanaxadiol 6-hydroxylase, *CYP716A52v2* β-amyrin 28-oxidase

freeze-dried, and resuspended within 5 ml n-hexane. Then, the mixture was analyzed by HPLC. The analysis was performed on a Phenomenex Prodigy C18 analytical column (5 μm, 150 × 3.2 mm) at 30 °C and 210 nm. Elution was carried out with methanol:$H_2O$ (3:1) at 1.0 ml/min. The contents of β-amyrin were calculated from the ratio of the peak area of the respective compound to that of the standard.

## Results

### Establishment of βAS-RANi hairy roots

The *βAS*-RNAi vector was constructed as above-described and transformed into *A. rhizogenes* A4. RNAi hairy root lines were induced by infection of 3–5-mm-thick ginseng disks with the positive A4.

After cultivation for 30–50 days, hairy roots started to appear at the infected sites; single root tips were picked off and subcultured on fresh MS medium with cefotaxime and kanamycin for several generations, until the bacteria were eliminated. Hairy root originated from a single root tip was considered as an independent clone. After establishment of *P. ginseng* hairy root clones, three control root lines and five RNAi lines were randomly selected among the generated 25 control and 36 RNAi lines, and adapted to liquid medium and maintained for more than eight generations before further analysis. RNAi lines were validated by PCR and sequencing. Furthermore, quantitative real-time PCR was adopted to evaluate the effect of RNAi on expression of *βAS* genes (*PNY1* and *PNY2*). As shown in Fig. 2, both genes were significantly down-regulated in the RNAi lines, in comparison with controls. In the following experiments, these control and RNAi lines were used. The amplified sequence from *PNY1* for RNAi has 87 % identity with *PNY2* at nucleotide level. Therefore, this level of sequence similarity is enough to trigger RNAi at least in this study. It would be interesting to study on the threshold level of sequence similarity for RNAi.

### Chemical analysis

HPLC analysis revealed that the level of ginsenoside $R_o$ was markedly reduced in RNAi lines, when compared with controls (Fig. 3), suggesting the important role of *βAS* in biosynthesis of ginsenoside $R_o$, the only one oleanane-type ginsenoside identified up to date.

To assess the influence of *βAS*-RNAi on ginsenoside production, levels of total ginsenosides and the typical dammarane-type ginsenoside $Rb_1$ and $Rg_1$ were analyzed and compared between control and RNAi lines. Compared to controls, total ginsenoside levels in RNAi lines were significantly enhanced (Fig. 3). Levels of ginsenoside $Rb_1$ and $Rg_1$ were also obviously increased. Furthermore, β-amyrin and dammarenediol in RNAi lines were quantitatively assayed. To some extent, the β-amyrin level was decreased, while the dammarenediol level was increased in RNAi lines (Fig. 4).

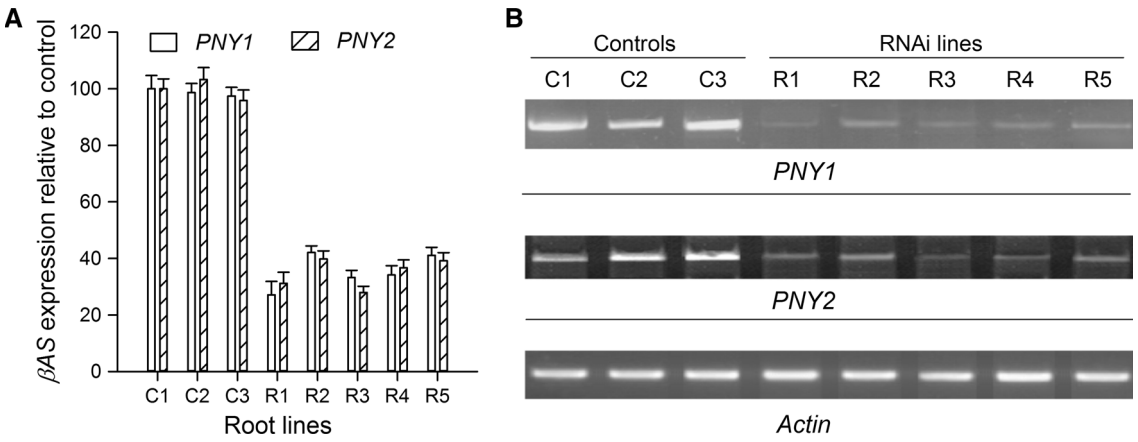


**Fig. 2** Analysis on expression of *βAS* genes (*PNY1* and *PNY2*) in 25-day-old root *lines* by quantitative real-time PCR. Three control *lines* and five RNAi *lines* were selected for the analysis. Each *histogram* represents the mean relative level from triplicate cultures expressed mean ± SD (standard deviation). Each culture was independently analyzed. The mean level of control *line* C1 is arbitrarily set to 100, the others are expressed as relative values to this level

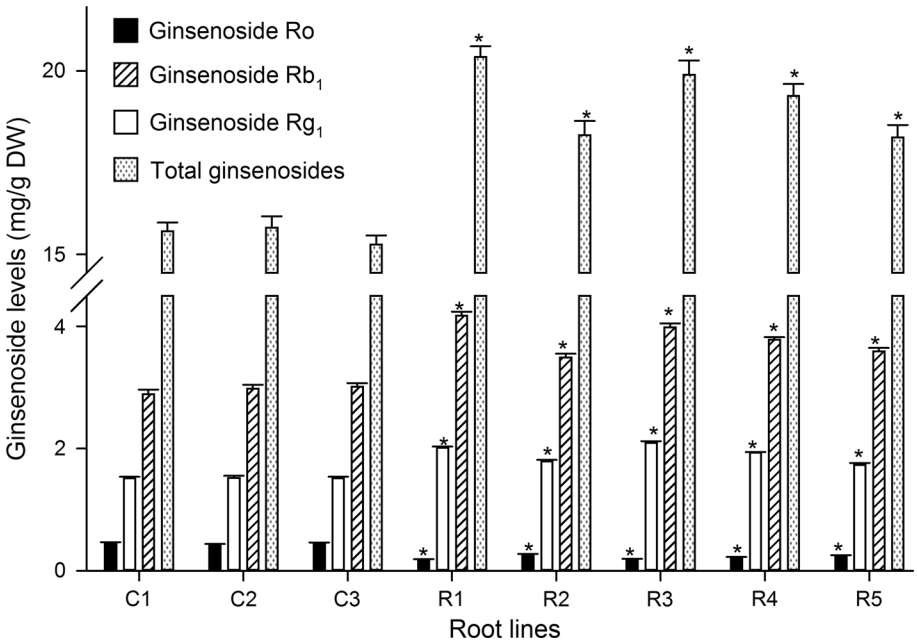


**Fig. 3** Comparison of ginsenoside levels between control and RNAi *lines*. Each *histogram* represents the mean level (±SD) from triplicate cultures. Each culture was independently analyzed. An *asterisk* above the *bar* indicates a statistical difference from the mean of controls, determined by a Dunnett's statistical test at $P \leq 0.05$

## Expression of key genes involved in ginsenoside synthesis

To further elucidate the mechanism underlying the improvement of dammarane-type ginsenoside level by *βAS*-RNAi, analysis on expression of key genes involved in ginsenoside biosynthesis was carried out by qPCR. The results are presented in Table 2. The expression of key genes (dammarenediol, protopanaxadiol, and protopanaxatriol synthases) involved in dammarane-type ginsenoside synthesis was significantly up-regulated. The β-amyrin oxidase gene was down-regulated. The expression of squalene synthase and cycloartenol synthase genes were not obviously changed.

## Growth

The RNAi hairy roots exhibited a typical growth pattern. When compared to control, RNAi line did not exhibit significant growth difference (Fig. 5). Moreover, these hairy roots lines had no obvious morphological difference.

## Elicitation

After a 4-day elicitation by MeJA, *βAS* expression could be enhanced both in non-RNAi lines and in RNAi lines (Fig. 6); however, the enhancement extent of RNAi lines is slightly lower than that of non-RNAi lines with a statistical difference. This indicated that MeJA-elicitation could not

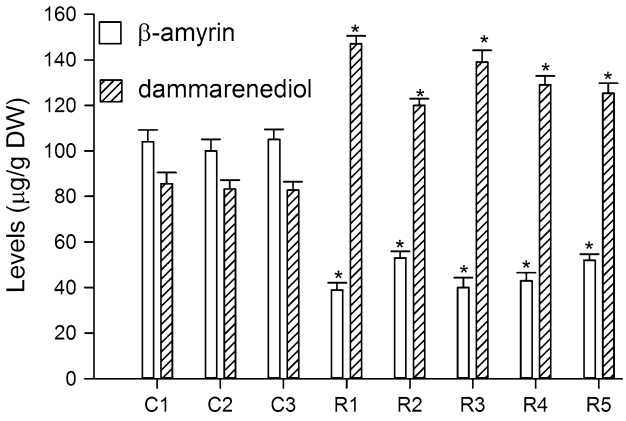


**Fig. 4** Quantitative determination of β-amyrin and dammarenediol in control and RNAi *lines* cultured for 25 days. Each *histogram* represents the mean level (±SD) of triplicate cultures. Each culture was independently analyzed. An *asterisk* above the *bar* indicates a difference from the mean of controls, determined by a Dunnett's statistical test at $P \leq 0.05$

completely counteract the effect of RNAi. Associated with the enhancement of *βAS* expression, ginsenoside Ro levels were also improved in both types of hairy roots.

## Discussion

In ginsenoside synthesis pathway, the roles of squalene synthase, 2,3-oxidosqualene synthase, and dammarenediol synthase genes have been evaluated (Han et al. 2006, 2010; Lee et al. 2004). In the present study, functional analysis of *βAS* gene in ginsenoside biosynthesis was performed by RNAi. RNAi of *βAS* in *P. ginseng* hairy roots resulted in down-regulation of β-amyrin, oleanane-type ginsenoside ($R_o$) levels as well as up-regulation of levels of total ginsenosides and dammarane-type ginsenosides ($Rb_1$ and $Rg_1$), indicating an important role of βAS in ginsenoside biosynthesis (Figs. 2, 3, 4). Since dammarenediol contests the same precursor with β-amyrin (Fig. 1), the enhancement on levels of dammarenediol and dammarane-type ginsenosides ($Rb_1$ and $Rg_1$) by *βAS*-RNAi was not surprising. The up-regulation of key genes involved in biosynthesis of dammarane-type ginsenosides (dammarenediol, protopanaxadiol, and protopanaxatriol synthases) and the enhancement of dammarane-type ginsenoside precursor dammarenediol (Fig. 4) made the improvement of dammarane-type ginsenoside contents reasonable. Since β-amyrin-derived compound synthesis pathway represents a competitive branch with that of dammarane-type ginsenoside, it is possible that *βAS*-RNAi led to an increased metabolism flux toward dammarane-type ginsenosides. Additionally, the down-regulation of β-amyrin oxidase may be caused by the decrease of β-amyrin level, given that this enzyme is of inducible-type. Although dammarane-type ginsenosides represent the majority of total ginsenosides, total ginsenoside content was markedly elevated by *βAS*-RNAi to a level that could not be explained by the decrease extent of ginsenoside $R_o$ level and the increase extent of dammarenediol level. This possibly implied that β-amyrin is not only used for the biosynthesis of ginsenoside $R_o$ and other β-amyrin-derived compounds should be present in *P. ginseng*. Further chemical constituent analysis targeting at discovering β-amyrin-derived compounds in ginseng would be helpful to further authenticate this speculation. It has been observed that amyrins are also involved in the biosynthetic pathways of other biologically active compounds besides ginsenosides such as avenacine, centellosides, and glycyrrhizin (Vázquez et al. 2012). Squalene synthase represents the regulatory valve controlling the total metabolism flux toward the oleanane-type ginsenoside synthetic pathway and

**Table 2** Expression of key genes involved in ginsenoside biosynthesis

| Root lines | Relative expression of genes[a] | | | | | |
|---|---|---|---|---|---|---|
| | Squalene synthase | Dammarenediol synthase | Protopanaxadiol synthase | Protopanaxatriol synthase | β-Amyrin oxidase | Cycloartenol synthase |
| C1 | 1.00 ± 0.05 | 1.00 ± 0.03 | 1.00 ± 0.03 | 1.00 ± 0.05 | 1.00 ± 0.06 | 1.00 ± 0.07 |
| C2 | 0.97 ± 0.04 | 1.03 ± 0.07 | 0.95 ± 0.05 | 1.02 ± 0.07 | 0.99 ± 0.04 | 1.04 ± 0.06 |
| C3 | 1.05 ± 0.08 | 0.99 ± 0.06 | 0.96 ± 0.04 | 1.03 ± 0.04 | 0.94 ± 0.05 | 1.03 ± 0.04 |
| R1 | 0.99 ± 0.04 | 1.71 ± 0.08 | 1.69 ± 0.02 | 1.66 ± 0.04 | 0.51 ± 0.03 | 1.05 ± 0.06 |
| R2 | 0.97 ± 0.06 | 1.41 ± 0.05 | 1.32 ± 0.06 | 1.48 ± 0.08 | 0.63 ± 0.06 | 0.99 ± 0.03 |
| R3 | 1.01 ± 0.07 | 1.55 ± 0.03 | 1.66 ± 0.05 | 1.63 ± 0.02 | 0.55 ± 0.07 | 1.06 ± 0.07 |
| R4 | 0.98 ± 0.03 | 1.58 ± 0.03 | 1.62 ± 0.08 | 1.60 ± 0.05 | 0.54 ± 0.05 | 1.02 ± 0.04 |
| R5 | 0.94 ± 0.06 | 1.44 ± 0.05 | 1.43 ± 0.04 | 1.37 ± 0.07 | 0.61 ± 0.03 | 0.99 ± 0.02 |

[a] The mean expression level of C1 is arbitrarily set to 1.00, and the others are expressed as values relative to this level

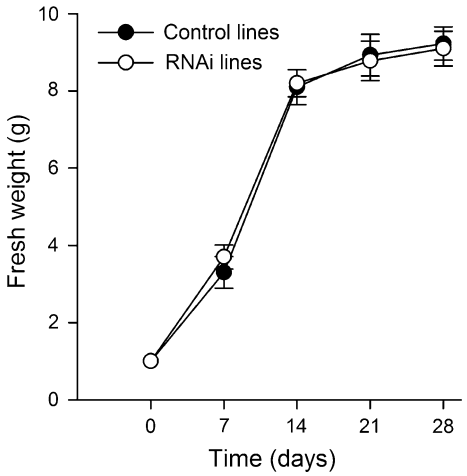


**Fig. 5** Growth of control and RNAi root *lines*. Each datum point represents the mean level (±SD) from selected lines of triplicate cultures. Each culture was independently analyzed. Statistical difference was determined by a Dunnett's statistical test at $P \leq 0.05$

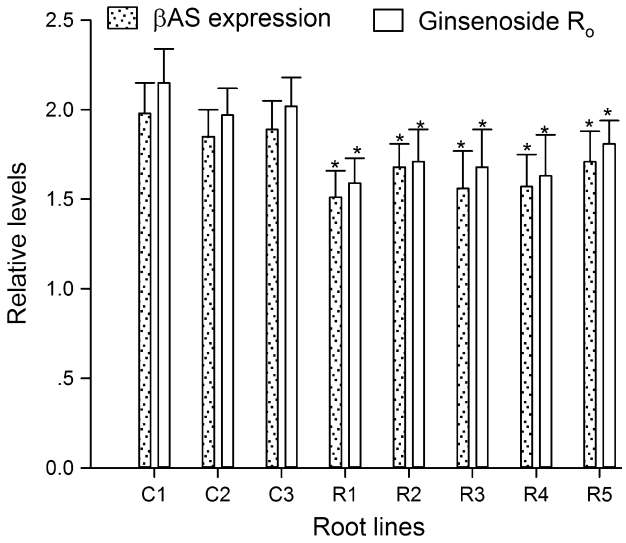


**Fig. 6** Effects of MeJA on βAS expression and olenane-type ginsenoside $R_o$ levels after a 4-day elicitation. Levels were expressed as relative expression levels of elicited *lines* to the corresponding controls (without elicitation). Each *histogram* represents the mean level (±SD) from triplicate experiments. Each culture was independently analyzed. An *asterisk* above the *bar* indicates a statistical difference from the mean of non-RNAi *lines* (C1, C2, and C3), determined by a Dunnett's statistical test at $P \leq 0.05$

other competitive pathways including dammarane-type ginsenoside and sterol synthetic pathways. Cycloartenol synthase is the key enzyme guiding the metabolism flux into sterol anabolism (Fig. 1). The expression levels of these two genes are not significantly changed in RNAi lines (Table 2), indicating the total metabolism flux and the branched flux for sterol are not significantly changed. Because sterol synthesis is primary metabolism but ginsenoside synthesis is secondary metabolism, we could speculate that, when redundant, the metabolism flux prefers to flow into secondary metabolism.

Moreover, the results in this study showed that *βAS*-RNAi had no significant effects on the vitality of *P. ginseng* hairy roots (Fig. 6). This indicated that *βAS* is not involved in growth of *P. ginseng* hairy roots. Likewise, it has been shown by a βAS-deficient mutant of *Avena strigosa* that βAS is not required for normal growth and development despite its essentiality for saponin biosynthesis (Haralampidis et al. 2001). Therefore, primary metabolism should be βAS independent.

MeJA has been reported to enhance *βAS* expression and accumulation of triterpene saponin (Hayashi et al. 2003; Liu et al. 2009; Lu et al. 2001; Shabani et al. 2010). However, other reports conflict with these results (Aoyagi et al. 2001; Kim et al. 2004). The present result is in agreement with the former view, since the *βAS* expression was up-regulated by MeJA treatment over a prolonged time period (1–7 days post treatment). The up-regulation of *βAS* expression was accompanied by an enhancement of ginsenoside Ro level, indicating an important role of *βAS* in oleanane-type ginsenoside biosynthesis on the other side.

Since the first report on enzymatic synthesis of β-amyrin (Corey and Ortiz de Montellano 1967), *βAS* has been cloned and identified in several plant species other than *P. gineng* (Kushiro et al. 1998), including *Glycyrrhiza glabra* (Hayashi et al. 2001), *Euphorbia tirucall* (Kajikawa et al. 2005), *Saponaria vaccaria* (Meesapyodsuk et al. 2007), *Gentiana straminea* (Liu et al. 2009), *Glycyrrhiza uralensis* (Shen et al. 2009), *Arabidopsis thaliana* (Shibuya et al. 2009), and *Aralia elata* (Wu et al. 2012). Furthermore, there is also another evidence for the involvement of *βAS* in saponin biosynthesis, namely reduction of saponin accumulation by the silence of β-amyrin synthase gene expression in soybean (Takagi et al. 2011). Nevertheless, more works are needed to elucidate the details on how β-amyrin is diverged into different saponins.

## Conclusion

Here we evaluated the roles of *βAS* in ginsenoside biosynthesis in *P. ginseng* by RNAi. The results showed that the RNAi-mediated inhibition of *βAS* decreased levels of β-amyrin and oleanane-type ginsenoside $R_o$, but increased the level of total ginsenosides. Dammarane-type ginsenosides represent the majority of total ginsenosides. Levels of the typical dammarane-type ginsenosides $Rb_1$ and $Rg_1$ were up-regulated. The key genes involved in dammarane-type ginsenoside synthesis were up-regulated including dammarenediol, protopanaxadiol, and protopanaxatriol synthases, accounting for the elevation of dammarane-type ginsenosides and total ginsenosides. The

MeJA-mediated up-regulation of *βAS* expression was accompanied by enhancement of ginsenoside Ro level, providing positive evidence on involvement of *βAS* in oleanane-type ginsenoside biosynthesis. These results showed that *βAS* plays an important role in oleanane-type ginsenoside synthesis and in metabolism flux allocation. The information will contribute to our understanding of ginsenoside biosynthesis.

**Author contribution statement** Shoujing Zhao and Luquan Ren Designed the research. Che Zhao, Tianhui Xu, Qian Wang, and Bo Dou performed the experiments. Yanlong liang, collected and analyzed data, and wrote the manuscript.

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**Conflict of interest** The authors declare that they have no conflict of interest.

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