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# Functional characterization of squalene epoxidase and NADPH-cytochrome P450 reductase in *Dioscorea zingiberensis*

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ABSTRACT

*Dioscorea zingiberensis* is a perennial medicinal herb rich in a variety of pharmaceutical steroidal saponins. Squalene epoxidase (SE) is the key enzyme in the biosynthesis pathways of triterpenoids and sterols, and catalyzes the epoxidation of squalene in coordination with NADPH-cytochrome P450 reductase (CPR). In this study, we cloned *DzSE* and *DzCPR* gene sequences from *D. zingiberensis* leaves, encoding proteins with 514 and 692 amino acids, respectively. Recombinant proteins were successfully expressed *in vitro*, and enzymatic analysis indicated that, when SE and CPR were incubated with the substrates squalene and NADPH, 2,3-oxidosqualene was formed as the product. Subcellular localization revealed that both the DzSE and DzCPR proteins are localized to the endoplasmic reticulum. The changes in transcription of *DzSE* and *DzCPR* were similar in several tissues. *DzSE* expression was enhanced in a time-dependent manner after methyl jasmonate (MeJA) treatments, while *DzCPR* expression was not inducible.

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## 1. Introduction

*Dioscorea zingiberensis* C. H. Wright is a traditional medicinal herb indigenous to China. The main medicinal ingredients of *D. zingiberensis* are steroidal saponins such as dioscin and diosgenin. Many different bioactivities are associated with the use of steroidal saponins, including anti-inflammation [1], hypolipidemic [2], and anti-proliferation [3] activities. However, the amount of these highly valued products in plants is low, which has hindered further research. Compared with extraction of steroidal saponins from plants, reconstruction of biosynthetic pathways in heterologous microbial hosts presents an attractive alternative approach to provide larger quantities of the product [4]. In recent years, some genes involved in diosgenin biosynthesis have been identified [5], although the specific details of the biosynthetic pathway remain unknown. Therefore, further research is necessary to identify the relevant enzymes and their functions.

Plant triterpenes and sterols are mainly synthesized via the cytosolic mevalonic acid (MVA) pathway [6]. Two farnesyl pyrophosphate (FPP) molecules are coupled head-to-head to form squalene by squalene synthase (SS) [7]. Then, squalene is formed to 2,3-oxidosqualene through an epoxidation catalyzed by squalene epoxidase (SE) (Fig. 1) [8]. 2,3-Oxidosqualene is cyclized into primarily skeletons by the action of oxidosqualene cyclases (*OSCs*), such as lanosterol in animals and fungi, and cycloartenol, dammarenediol, and β-amyrin in plants [9]. After cyclization, triterpenes and sterols are usually further modified by cytochrome P450s, acetyltransferases and glycosyltransferases [10]. SE is a rate-limiting enzyme that regulates the downstream pathways for the production of triterpenoids and phytosterols [11].

The epoxidation of squalene also requires the participation of NADPH-dependent cytochrome P450 reductase (CPR) [12]. CPR transfers electrons from NADPH via FAD and FMN to the heme group of the P450 protein. Squalene epoxidase does not contain a heme group, but is dependent on CPR to insert oxygen of the hydroperoxide to squalene [13]. CPR paralogs in plants range from one to three, and the diversity of the CPRs indicates the complexity of their functions [14]. Based on the sequences of their N-terminal hydrophobic regions, the CPRs are divided into class I and class II categories [15].

SEs and CPRs are involved in a broad range of plant development and secondary metabolite biosynthetic reactions. *Arabidopsis* squalene epoxidase 1 (*sqe1*) mutants reduce root and hypocotyl elongation and fail to produce viable seeds [16]. Squalene

*Abbreviations:* SE, squalene epoxidase; CPR, NADPH-cytochrome P450 reductase; RACE, Rapid-amplification of cDNA ends; GC-MS, gas chromatography-mass spectrometry; PEG, polyethylene glycol; MeJA, methyl jasmonate.

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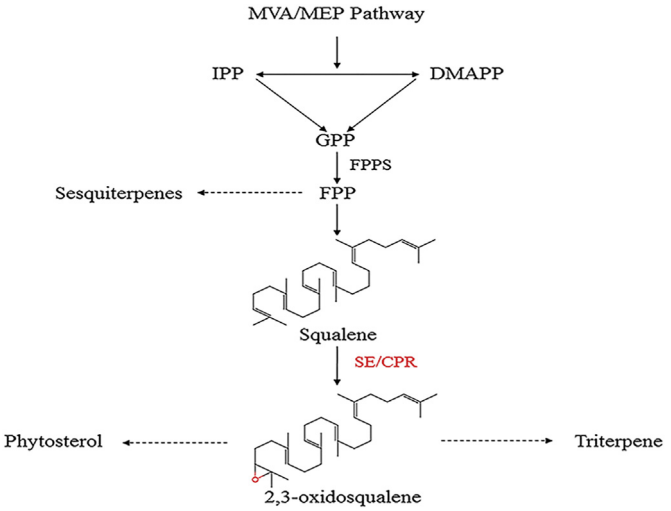


**Fig. 1. An overview of terpenoids and sterols biosynthesis pathway.**
Biosynthesis of terpenoids and sterols in *D. zingiberensis*. Red box indicates the epoxidation of squalene.

epoxidase 3 contributes to the bulk SE activity in *Arabidopsis* and is essential for embryo development [17]. Silencing of *PgSQE1* reduces ginsenoside production, but leads to increased phytosterol accumulation in *Panax ginseng* [11]. As protein partners of cytochrome P450s, CPRs participate in various biomolecular synthesis pathways, such as those producing fatty acids, hormones, pigments, and various defensive compounds [18]. Thus, characterization of SEs and CPRs is crucial to the understanding of plant growth, development, metabolism, and other aspects of plant biology. In this report, we provide an in-depth understanding of the synthesis mechanisms of terpenoids and sterols in *D. zingiberensis* and a means for improving the yields of downstream products such as diosgenin.

## 2. Materials and methods

### 2.1. Planting conditions

*Dioscorea zingiberensis* specimens were grown in coconut soil and cultured at 25 ± 2 °C under natural light-dark conditions.

### 2.2. Cloning and sequence analysis of DzSE and DzCPR

Total RNA was isolated from *D. zingiberensis* leaves using an RNA extraction kit (Bioteke, China). First-strand cDNA was synthesized with 1 μg of RNA using the HiScriptII 1st Strand cDNA Synthesis Kit (Vazyme, China) or the SMARTer RACE cDNA Amplification Kit (Clontech, USA), according to the manufacturer's instructions. The synthesized cDNA was diluted to 10 ng/μL with RNase-free water and dispensed for later use.

The degenerate primers (Table 1) were designed based on the conserved regions of plant SE and CPR gene sequences and were used to amplify core fragments. The amplified products were detected on 1.2% agarose gels stained with GelRed (Biotium, USA) and sequenced. The 5′- and 3′-end cDNA sequences were obtained using two rounds of nested PCR with the appropriate specific primers (Table 1). The full-length cDNAs were amplified, purified from agarose gels with the AxyPrep DNA Gel Extraction Kit (Axygen, USA), and ligated into the pMD19-T cloning vector followed by sequencing.

Sequence alignment and homology search analysis of the DzSE and DzCPR proteins were performed using the Basic Local Alignment Search Tool (BLAST; http://www.ncbi.nlm.nih.gov/BLAST). Physicochemical properties, hydrophobicity, hydrophilicity, and transmembrane regions of the deduced proteins were predicted using the ExPASy ProtParam tool (http://web.expasy.org/protparam) and TMHMM software (http://www.cbs.dtu.dk/services/TMHMM-2.0). The sequences were collected and multiple alignments were performed in the ClustalX program. A phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis version 5 (MEGA5) software using the neighbor-joining algorithm method at 1000 bootstrap replicates.

**Table 1**
List of primers used in the study.

| Primer names | Sequence 5′→3′ |
|---|---|
| *Degenerated* | |
| SE-dF | GATGTDDCTGGRAGRAGCTT |
| SE-dR | GTRGAWGCYACDGGCTTRCG |
| CPR-dF | KGGYCTTGGAGATGATGATC |
| CPR-dR | CATRCCYTTRCGATCACCA |
| *RACE* | |
| 5′SE-Outer | GATAGAACAAGATTGGGGAGGG |
| 5′SE-Inner | TCTTCACCTGCTCGGGTCTTGT |
| 5′CPR-Outer | TCCAGTCCCAGCAATGTCAAAC |
| 5′CPR-Inner | GTAAGGGGTAGATGCACCAGAT |
| 3′SE-Outer | CCATTGACAAGGGCAGCATAAG |
| 3′SE-Inner | CTGGAGGAGGAATGACTGTGGC |
| 3′CPR-Outer | GGGATAGAACTTGGCCATGCT |
| 3′CPR-Inner | TCTCTGAGCTGATTGTTGCCT |
| SE-ORF-F | ATGATGCTTTCCGAGTATCTC |
| SE-ORF-R | TTTAGTAGGCGGCGGCGGT |
| CPR-ORF-F | ATGAAGCTCTCGGCGCTGGA |
| CPR-ORF-R | CCAGACATCACGAAGATATC |
| *Expression* | |
| SE-pMal-F | GAGGGAAGGATTTCAGAATTCATGATGCTTTCCGAGTATCTC |
| SE-pMal-R | CAAGCTTGCCTGCAGGTCGACTTTAGTAGGCGGCGGCGGT |
| CPR-pET32a-F | GACGACGACGACAAGGCCATGGATGTCGACGATGGGAAGA |
| CPR-pET32a-R | GGTGGTGGTGGTGGTGCTCGAGCCAGACATCACGAAGATATC |
| *Subcellular* | |
| SE-pCXUN-F | GGGATCCCCAATACTCCTAGGATGATGCTTTCCGAGTATCTC |
| SE-pCXUN-R | GCCCTTGCTCACCATACTAGTTTAGTAGGCGGCGGCGGT |
| CPR-pCXUN-F | GGGATCCCCAATACTCCTAGGATGAAGCTCTCGGCGCTGGA |
| CPR-pCXUN-R | GCCCTTGCTCACCATACTAGTCCAGACATCACGAAGATATC |
| *Real-time* | |
| SE-qF | GGCATGTTGTGTTAGCAGATC |
| SE-qR | CATGCAATTCTCCAGGGATCT |
| CPR-qF | GTGGAAGAGGCTGAAAGGC |
| CPR-qR | GTCGGCATATCGGGAAAGTG |
| GAPDH-qF | TTTGGTGAAAAGTCAGTCGCA |
| GAPDH-qR | TCGGAGCAGAAATGACAACCT |

### 2.3. Expression of recombinant proteins in Escherichia coli

The entire DzSE coding region and an N-terminal-truncated DzCPR cDNA fragment were subcloned into the pMal-c2x and pET-32a vectors, respectively, by homologous recombination using the ClonExpress II One Step Cloning Kit (Vazyme, China). The vector constructs were transformed into Transetta (DE3) competent cells and single colonies were selected, inoculated into liquid LB medium containing 100 mg/L ampicillin, and shaken overnight at 37 °C. The cultures were transferred into 1 L of fresh liquid LB medium containing 100 mg/L ampicillin and shaken at 37 °C at 180 rpm until the OD600 reached 0.6. Expression of DzSE and DzCPR was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) for 16 h at 18 °C with shaking at 160 rpm. The bacteria were harvested by centrifugation at 3000×*g* for 10 min at 4 °C and resuspended in 50 mM Tris-HCl. The cells were disrupted by sonication and then centrifuged at 15000×*g* for 20 min at 4 °C. Recombinant DzSE was purified using an MBPTrap HP affinity column and eluted with

10 mmol maltose in 50 mM Tris·HCl pH 7.5. The DzCPR supernatant was loaded on a Ni-NTA agarose column and eluted with 50 mM Tris-HCl buffer containing 250 mM imidazole. The recombinant proteins were detected via 10% (w/v) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein concentration was assessed using the Bradford assay and the recombinant proteins were stored at −20 °C.

### 2.4. Enzyme activity assay

DzCPR enzymatic activity was measured by spectrophotometry at 550 nm. The reaction contained 1 μg of protein and 50 mM cytochrome c in 1 mL of 0.3 M potassium phosphate buffer (pH 7.6) at 25 °C. The change in the absorbance was recorded after adding 10 μL of 10 mM NADPH solution. Enzyme activity was detected at 1-min intervals and measured by cytochrome c reduction [19]. To evaluate the kinetic parameters of cytochrome c, various amounts of cytochrome c were added to the reactions, while the concentration of NADPH remained fixed at 50 μM. The kinetic parameters of NADPH were determined using the same principle. The Michaelis constant $K_m$ and $V_{max}$ were calculated using nonlinear Michaelis-Menten plots using GraphPad Prism 7 software.

DzSE enzymatic activity was determined by the synthesis of 2,3-oxidosqualene. The reaction mixture contained 0.2 mg squalene (Sigma-Aldrich, USA), 0.5 mg recombinant DzSE protein, 0.5 mg recombinant DzCPR protein, 1 mM FAD, 1 mM NADPH, Triton X-100 (0.5%), and 50 mM Tris-HCl (pH 7.5) in a total volume of 2 mL. The mixture was extracted with hexane for three times after incubation at 25 °C overnight. The extracts were analyzed by gas chromatography-mass spectrometry (GC-MS).

### 2.5. Subcellular localization

The full-length *DzSE* and *DzCPR* open reading frames (ORFs) were cloned into a modified plant expression vector *pCXUN-GFP* by homologous recombination using the ClonExpress II One Step Cloning kit (Vazyme, China) to allow expression of protein fused with green fluorescent protein (GFP). The construct vectors *DzSE-GFP* and *DzCPR-GFP* were co-transformed into the rice protoplasts by PEG-mediated [20]. Transformed protoplasts were visualized using an Olympus FV1000 confocal microscope.

### 2.6. Tissue-specific expression and elicitor treatment

To determine tissue expression profiles of *DzSE* and *DzCPR*, total RNA was extracted from young leaves, mature leaves, stems, flowers, and rhizomes of *D. zingiberensis*, and reverse transcribed into cDNA. qRT-PCR primers (Table 1) were designed and the reactions were performed using Fast Start Universal SYBR Green Master (Roche, Germany) with a CFX96 real-time system (Bio-Rad, USA). The GAPDH housekeeping gene was used as an internal control. To explore the effects of elicitor treatment, young leaves were harvested after treatment with 50 μM methyl jasmonate (MeJA) at 6, 12, 24, 48, and 72 h for RNA isolation, including a control. All samples were examined in triplicate and experimental data were analyzed using the $2^{\Delta\Delta Ct}$ method.

## 3. Results

### 3.1. Cloning and analysis of DzSE and DzCPR gene sequences

A core fragment of each of SE and CPR gene was amplified from leaf cDNA using degenerate primers. Specific primers were then designed according to the obtained core sequences to amplify the 5′- and 3′-terminal sequences. Fragments of SE and CPR genes were assembled into full-length cDNAs of 1927 and 2475 nucleotides, respectively. The SE and CPR coding regions contained 1542 and 2076 bp, encoding 514 and 692 amino acids, respectively.

The predicted molecular mass and theoretical isoelectric point (pI) of DzSE were 55.7 kDa and 8.83, respectively. Amino acid sequence analysis revealed that DzSE belongs to the PLN02985 super family and has a putative FAD-binding domain, which is an essential site for enzyme catalytic activity. Another major functional domain is an NAD (P)-binding site which is part of a Rossmann-fold to bind FAD and $NADP^+$ (fig. S1). The sequence NMRHPLTGGGMTV was conserved in the alignment and binds squalene as a substrate. Transmembrane domains were predicted at the N- and C-termini, suggesting that the DzSE protein is anchored to the membrane. The phylogenetic tree indicated that SEs were divided into true SEs and SE-like proteins. DzSE was classified as a true SE and clustered in the same subclade with SE isolated from *Chlorophytum borivilianum* (Fig. S3).

The calculated monomeric molecular weight and theoretical pI of DzCPR were 76.8 kDa and 5.49, respectively. Amino acid sequence alignment showed that DzCPR had three characteristic domains, the FAD-, FMN-, and NADPH-binding domains (Fig. S2). The conserved motif LGDDDQCIEDD in DzCPR was presumed to interact with P450s and cytochrome c. A linker region between the FAD- and FMN-binding domains promotes internal electron transfer and assists in the interaction of CPR with diverse P450s. The phylogenetic tree revealed that DzCPR belongs to CPR class I and was closed to CPR isolated from *Brachypodium distachyon* (Fig. S4).

### 3.2. Recombinant protein expression in E. coli

The entire DzSE ORF was integrated into the pMal-c2x expression vector containing the tac-promoter and the maltose-binding protein (MBP) sequence to generate a fusion protein. The constructed pMal-DzSE plasmid was transformed into Transetta (DE3) chemically competent cells and induced at a low temperature to reduce the formation of inclusion bodies. After induction, a band at approximately 98 kDa (55.7-kDa DzSE protein plus the 42.5-kDa maltose-binding protein) was only partially observed in the supernatant (Fig. S5A). To increase the solubility of the protein, 74 amino acids at the N-terminus of DzCPR were truncated to exclude a predicted transmembrane region. The remaining sequence was inserted into the pET-32a vector with expression under the control of the T7-promoter. SDS-PAGE revealed that the truncated DzCPR was 86.8 kDa (68.8 kDa DzCPR with an 18-kDa fusion tag) and was expressed in supernatant (Fig. S5B).

### 3.3. Identification of SE and CPR function

To demonstrate that SE and CPR were catalytically active in 2,3-oxidosqualene synthesis, we first confirmed CPR activity. DzCPR enzyme activity and kinetic parameters were measured via adjusting the concentration of NADPH or cytochrome c in an assay. In the assay reaction, cytochrome c was reduced after receiving electrons from NADPH via CPR. The $K_m$ and $V_{max}$ of DzCPR were $12.1 \pm 0.9$ μM and $10.7 \pm 0.2$ μmoL/min/mg protein for NADPH (Fig. 2A), and $15.9 \pm 1.3$ μM and $10.8 \pm 0.3$ μmoL/min/mg protein for cytochrome c (Fig. 2B), respectively.

DzSE function was determined by GC-MS analysis of the products from the incubation of purified recombinant SE and CPR proteins with squalene and NADPH. The GC results revealed that a new peak appeared at 11.66 min (Fig. 2C), which was identified as 2,3-oxidosqualene according to the characteristics of the primary ion peaks (Fig. 2D). However, 2,3-oxidosqualene was not detected in the extracts without addition of the CPR protein, even when the amount of exogenous squalene was increased.

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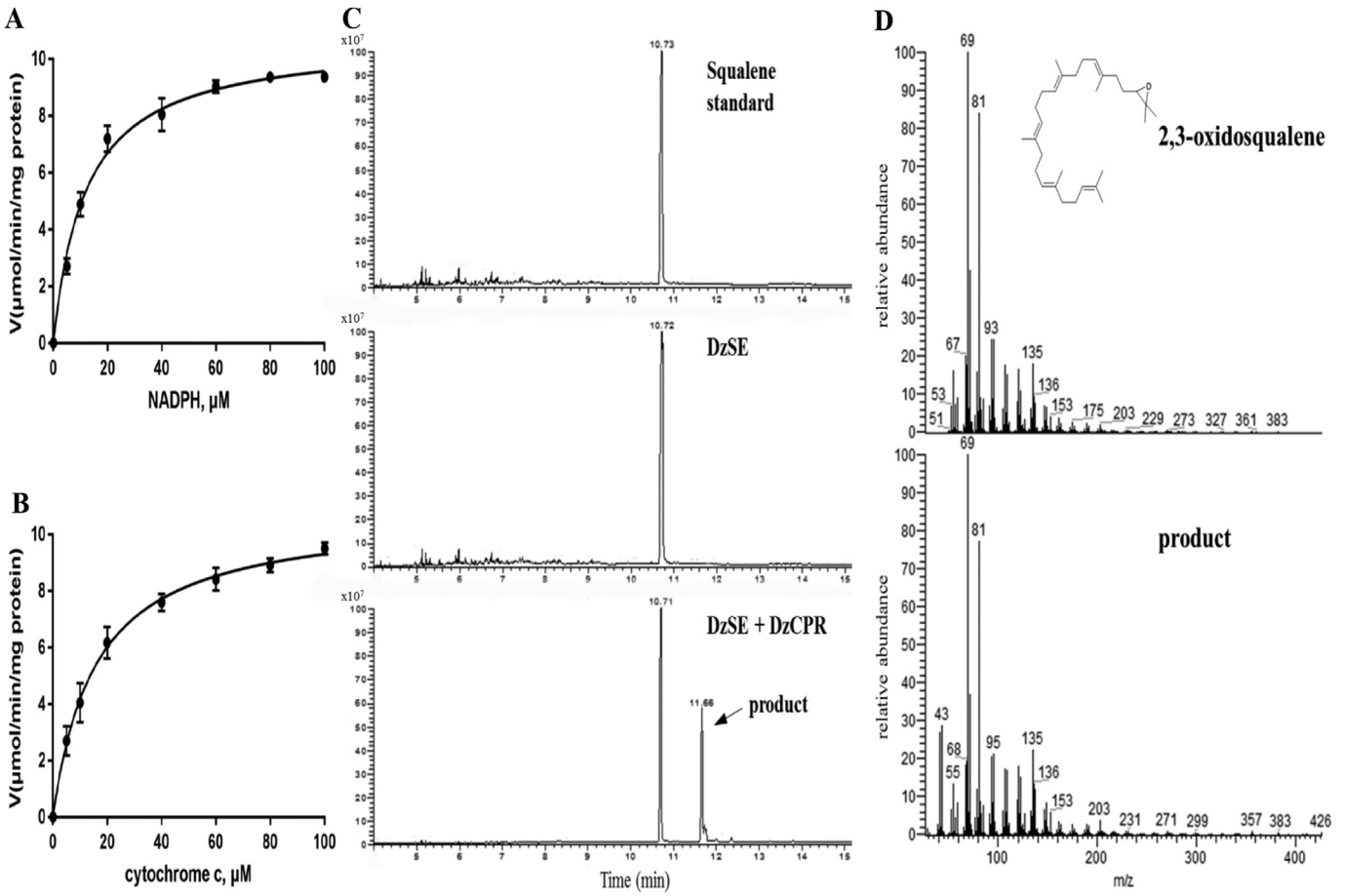


**Fig. 2. Enzyme activity of recombinant DzSE and DzCPR.**
(A) The kinetic constants of recombinant DzCPR for NADPHA (50 mM).
(B) The kinetic constants of recombinant DzCPR for cytochrome c (50 mM).
(C) GC results of the enzymatic reaction.
(D) MS results revealed the primary ion peak of the 2,3-oxidosqualene standard and the enzymatic product.

### 3.4. Subcellular localization analysis

To determine the subcellular localization of DzSE and DzCPR, GFP was fused to the C-terminus of both proteins. The fluorescence signal demonstrated that the GFP-fused SE and CPR proteins were both strictly localized to the endoplasmic reticulum (ER) of rice protoplasts, which was consistent with the location of ER organelle marker (Fig. 3). Different predicted transmembrane domains were discovered at the C-terminus of both proteins, which might act to anchor the enzyme to the ER membrane.

### 3.5. Tissue expression of DzSE and DzCPR and the response to MeJA

To study the patterns and levels of *DzSE* and *DzCPR* expression in tissues of *D. zingiberensis*, we isolated total RNA from leaves, stems, rhizomes, and flowers as template for qRT-PCR. The results of the qRT-PCR analysis demonstrated that both genes were expressed in specific organs, with the highest level of *DzSE* expression in young leaves followed by mature leaves, flowers, stems, and rhizomes. The highest level of *DzCPR* expression was found in mature leaves followed by flowers, young leaves, rhizomes, and stems (Fig. 4A). *DzSE* expression was markedly induced after treatment with MeJA, whereas *DzCPR* expression remained unchanged. *DzSE* transcript levels increased dramatically over time in response to MeJA, reaching a peak level around 48 h after induction, and subsequently decreased from 48 to 72 h after induction (Fig. 4B).

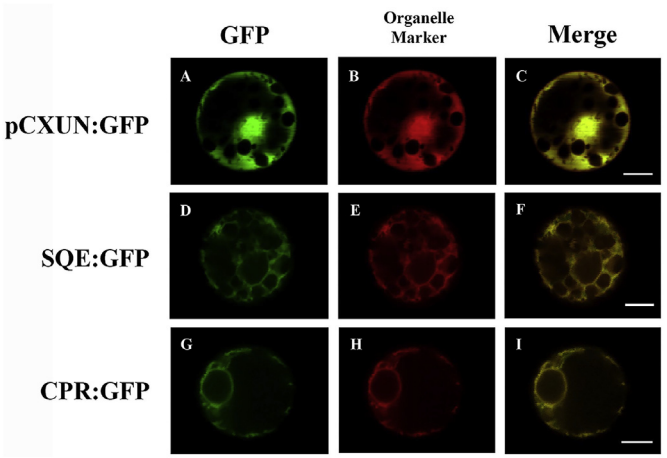


**Fig. 3. Subcellular localization of DzSE and DzCPR proteins.**
Subcellular localization of pCXUN-GFP (A–C), pCXUN-DzSE-GFP (D–E), and pCXUN-DzCPR-GFP (G–I) in rice protoplasts. Fluorescent GFP signals (A, D, G), marker (B, E, H), and merged images (C, F, I) are shown. Fluorescence was observed using a laser scanning confocal microscope. Bars = 5 μm.

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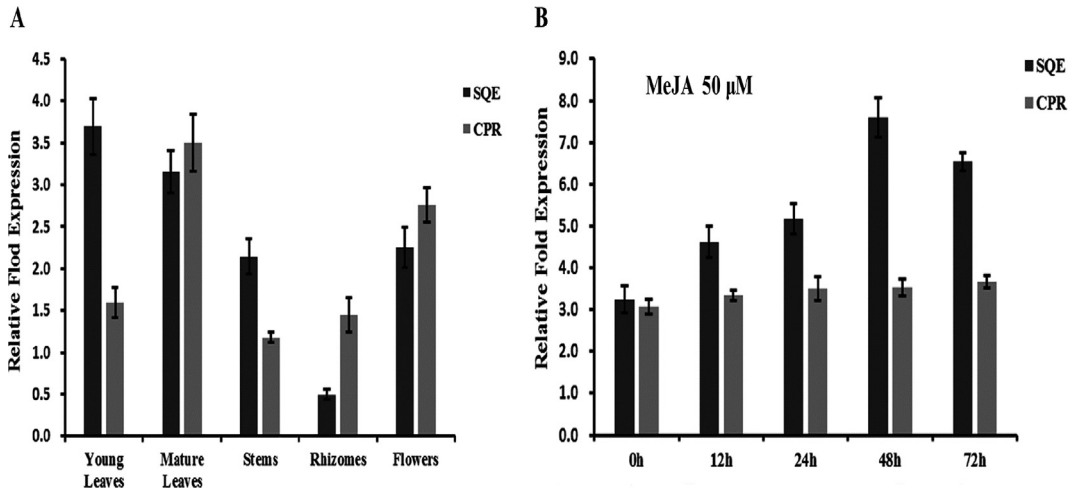


**Fig. 4. Tissues expression of *DzSE* and *DzCPR* genes and elicitor treatment.**
(A) Expression patterns of *DzSE* and *DzCPR* genes in tissues of *D. zingiberensis*.
(B) *DzSE* and *DzCPR* expression in leaves of *D. zingiberensis* treated with methyl jasmonate (50 μM).

## 4. Discussion

SE catalyzes the formation of a common precursor in the biosynthesis of triterpenoids and sterols and is recognized as one of the rate-limiting enzymes. SE catalyzes the formation of 2,3-oxidosqualene from squalene, and CPR transfers electrons from NADPH to complete the reaction. In this study, we successfully isolated full-length cDNAs for SE and CPR from *D. zingiberensis*. The deduced amino acid sequences for both genes confirmed that the SE protein contained an SE domain and two FAD/NAD (P)-binding domains, and that the CPR protein had the characteristics of NADPH-cytochrome reductases (i.e., FMN-, FAD-, and NADPH-binding domains). According to protein function and phylogenetic analyses, SEs are classified into true SE and SE-like proteins. DzSE was classified as a true SE, which means that it is likely to retain standard SE function. SE-like proteins appear to be exclusive to the *Brassicaceae*, and are not capable of restoring the function of a yeast SE mutant [21]. DzCPR belongs to CPR class I, the members of which possess a short stretch of amino acids at the N-terminus.

Because SE and CPR are membrane-bound proteins, they generally fail to fold into the correct conformation when expressed in *E. coli*. Removal of the transmembrane region of recombinant proteins can usually increase their solubility. Rat SE was successfully expressed in *E. coli* after truncation of membrane domain [22]. A truncated yeast CPR without the N-terminal transmembrane domain increased the expression of soluble protein [23]. Neither of the natural DzSE and DzCPR proteins were produced in soluble form using the pET-32a expression system. After optimization, the truncated pET-DzCPR was functionally expressed in *E. coli* and displayed a higher enzyme activity than the unmodified protein. However, the truncated SE remained insoluble using the same approach. Since maltose-binding protein improves the solubility and promotes the proper folding of its fusion partners [24], we replaced the pET-32a system with the pMal-c2x expression system. The full-length pMal-DzSE fusion protein was successfully expressed partially in the supernatant of *E. coli* cultures. 2,3-Oxidosqualene could only be detected when both the DzSE and DzCPR recombinant proteins were present in reaction mixtures. In the absence of any enzyme including NADPH, the epoxidation of squalene did not occur. This clearly suggests that both SE and CPR are directly involved in the catalysis of 2,3-oxidosqualene.

The synthesis of triterpenoids is usually associated with the ER. It has been proposed that the ER is responsible for transport and/or synthesis of terpenoids in plant oil gland cells [25]. The subcellular localization of yeast SE (Erg1p) has been reported to be in the ER and lipid droplets by immunostaining [26]. Two divergent CPR isoforms from hybrid poplar were targeted to the ER, whereas *Arabidopsis* P450 reductase 2 contains a high Ser: Thr ratio in its N-terminus and thus might be targeted to chloroplasts [15]. In this study, GFP fusion protein fluorescence signals revealed that both DzSE and DzCPR are anchored and retained on the ER membrane, which is consistent with predictions obtained using Euk-mPLoc 2.0 software. 2,3-Oxidosqualene is catalyzed by oxidosqualene cyclase and P450 enzymes into various terpenoids, and the ER provides a suitable oxidizing environment for the series of reactions.

The involvement of SE and CPR in the biosynthesis of 2,3-oxidosqualene is also investigated by the tissue expression profile analysis in the leaves, stems, flowers, and rhizomes. Both genes exhibited high levels of transcription in leaves, indicating that the leaves are the major organ in which the synthesis of 2,3-oxidosqualene occurs. Our previous report indicated that saponins mainly accumulated in the rhizomes rather than in the leaves [27]. Therefore, we speculate that saponins and their precursors are mainly synthesized in leaves and transferred into rhizomes for storage through stem vascular bundles, similar to the mechanism that occurs in *Achyranthus bidentate* [28]. MeJA is generally considered to modulate expression of genes involved in the biosynthesis of triterpene and sterol saponins [29]. In this study, the *DzSE* transcript level increased greatly in response to MeJA, and reached its maximum at 48 h. This indicates that MeJA has a transient upregulation effect on the synthesis and accumulation of 2,3-oxidosqualene. The elicitor had no significant influence on *DzCPR* expression, which was attributed to the categorization of *DzCPR* into CPR class I genes which are expressed constitutively, whereas CPR class II genes are transcribed in response to environmental stimuli such as stress or wounding [30]. The data suggest that DzSE functions specifically in 2,3-oxidosqualene biosynthesis, whereas CPR has a more general function *in vivo*.

In conclusion, we cloned and characterized the *DzSE* and *DzCPR* genes. Recombinant DzSE and DzCPR proteins were successfully expressed *in vitro* and purified. The fluorescence signals of GFP-fusion proteins indicated that both proteins are localized to the ER. The expression patterns of *DzSE* and *DzCPR* are related in plant

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tissues, and *DzSE* demonstrated a strong response to induction by MeJA.

## Conflicts of interest

The authors declare no conflicts of interest involving this article.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2019.01.010.

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