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Cloning and characterization of *MECS* and *HDS* genes from *Rauvolfia verticillata*

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Abstract

2-C-Methyl-D-erythritol-2,4-cyclodiphosphate synthase (MECS, EC: 4.6.1.12) and 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate synthase (HDS, EC: 1.17.4.3) are two genes involved in the methylerythritol phosphate (MEP) pathway, which catalyze the fifth and the sixth enzymatic reaction of MEP pathway, respectively. The full-length cDNA of MECS and HDS were cloned and characterized from *Rauvolfia verticillata* for the first time. The new cDNAs were designated as *RvMECS* and *RvHDS* and submitted to GenBank® to be assigned with an accession number: EU034699 and HQ659759, respectively. The full-length cDNA of *RvMECS* was 1021-bp, containing a 717-bp open reading frame which encoded a polypeptide of 238 amino acids with a calculated molecular mass of 25.4 kDa and an isoelectric point of 8.32. The full-length cDNA of *RvHDS* was 2645-bp, containing a 2220-bp ORF encoding 740 amino acids with a calculated molecular mass of 82.0kDa and an isoelectric point of 6.28. Bioinformatic analysis revealed that both *RvMECS* and *RvHDS* had extensive homology with *MECS*s and *HDS*s from other plant species and contained a conserved transit peptide for plastids. The phylogenetic analysis indicated that *RvMECS* and *RvHDS* belonged to plant *MECS*s family and *HDS*s family respectively. Quantitative PCR showed that expression level of *RvMECS* was highest in flowers followed by stems, roots, leaves and fruits. Whereas *RvHDS* expression level was highest in flowers followed by leaves, stems, fruits and roots. Expression profile analysis revealed that *RvMECS* expression was up-regulated by exogenous elicitors including methyl jasmonate and UV. However, expression of *RvHDS* was inhibited by elicitors. The present study will be helpful to understand more about the function of the two MEP genes at the level of molecular genetics.

Keywords: *Rauvolfia verticillata*; *MECS* gene; *HDS* gene; Cloning; MEP pathway; Expression profile.
Abbreviations: MECS- 2-C-Methyl-D-erythritol-2,4-cyclodiphosphate synthase; HDS- 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate synthase; MEP- methylerythritol phosphate; MeJA- methyl jasmonate; ABA- abscisic acid; ASA- acetyl salicylic acid; 6-BA- 6-benzyl aminopuine; NAA- α-Naphthalene acetic acid; TIAs- Terpenoid Indole Alkaloids.

Introduction

Rauvolfia verticillata is a rare medicinal shrub belonging to the family Apocynaceae, which is the main source of reserpine and ajmalicine in China (Li and Ting, 1962).Terpenoid Indole Alkaloids (TIAs) constitute one of the largest groups of natural products, providing many pharmacologically active compounds such as ajmalicine and reserpine. Especially, reserpine is of most interest because of its application in hypertension and cardiac disorders treatment (Anitha and Ranjitha, 2006). In pharmaceutical industries, reserpine is mainly extracted from the nature sources; however, the great demand cannot be met due to its low content. Even though the chemical synthesis of reserpine is possible, it is not feasible because of the high costs. Consequently, it is eager and necessary to find an alternative way to provide the source of pharmaceutical TIAs. Therefore, to map TIAs biosynthetic pathway in *R. verticillata* at the level of molecular genetics is a promising way to increase pharmaceutical TIAs production.The common precursors of TIAs, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are synthesized through the recently discovered plastidial methylerythritol 4-phosphate (MEP) pathway, which operates in eubacteria and plant plastids (Ramos-Valdivia et al., 1998). It has been confirmed that MEP pathway is essential for plastidial isoprenoid biosynthesis in plants (Rodríguez-Concepción, 2004). MEP pathway consists of seven enzymatic transformations originating form the conversion of pyruvate and glyceraldehyde 3-phophate (Figure 1). Two cDNAs involved in MEP pathway encoding 1-deoxy-d-xylulose 5-phosphate reductoisomerase (DXR) (Liao et al., 2007) and 1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase (HDR) have been cloned and characterized in the previous reports of our laboratory (Chen et al., 2010). MECS catalyzes the conversion of 4-diphosphocytidyl-2-C-methyl-D-erythritol 2-

Table 1. The nucleotide sequences of oligonucleotide primers

Primers	Orientation	Sequence (5'-3')
dfmecs	Sense	GAGGATGCGA(C/G)GCTCACTCTG
drmecs	Antisense	C(C/T)CCAAGACTGTC(A/T/C)ACCTTCTC
RvMECS3-1	Sense	GGCTACCAGACATAGGGCAA
RvMECS3-2	Sense	GGAAAGGGGCACCTTCTTC
RvMECS5-1	Antisense	CCCCAAGACTGTCGACCTTCTCATG
RvMECS5-2	Antisense	CCTGCCTCATGCATCAGCCTCACA
ffrvmecs	Sense	ACCCGGGGAAAGCCAAAGAC
frrvmecs	Antisense	CGCTCTAAGATATAGTAATTTTGC
fexRvMECS	Sense	CTTCGTTCTACTGCTCCACTTC
rexRvMECS	Antisense	AGAGACTGCGACTGCCTTC
dfhds	Sense	GATGG(T/C)TCTGT(T/A)C(T/C)(C/A)ATGTC(C/T/G/A)
drhds	Antisense	GGTCCATT(T/G/C)ACAAT(A/G)CA(G/A/T)CCC
RvHDS3-1	Sense	TTACTACAAGGTTGCAGATTG
RvHDS3-2	Sense	TATGTGTCATGCCCATCCT
RvHDS5-1	Antisense	CCACGTACAGAAACAGCCAAACGTGTTCC
RvHDS5-2	Antisense	GCCAGATCCTTAAACGGCATTCCAACCACC
ffrvhds	Sense	CGGATTCTTCTGTTCCGGAC
rfvhds	Antisense	GAGCTTTGGTGCATGATCTTACTC
fexRvHDS	Sense	TGACCACAACAGACACCAAGGATG
rexRvHDS	Antisense	GCAACTCGCAAAGCCACAGAAG
18SF	Sense	ATGATAACTCGACGGATCGC
18SR	Antisense	CTTGGATGTGGTAGCCGTTT
UPM	Sense	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGAGT CTAATACGACTCACTATAGGGC
NUP	Sense	AAGCAGTGGTATCAACGCAGAGT
Oligo (dT)17 primer	Antisense	GGCCACGCGTCGACTAGTAC (T) 17
M13 primer M4	Antisense	GTTTTCCCAGTCACGAC

phosphate (CDP-ME2P) to 2-C-methyl-D-erythritol 2,4-cyclodiphosphate (MECDP), which is the fifth reaction of MEP pathway. The depletion experiment in both *Escherichia coli* and *Bacillus subtilis* demonstrated that MECS had an early and significant impact on cell wall biosynthesis and leads to cell death ultimately (Campbell and Brown, 2002). In larger genomic scale hybridization studies, failure to insert a transposon into the *ispF* gene (gene encoding MECS enzyme in *E. coli*) also suggested MECS was essential for growth or survival of *Haemophilus influenzae* and *Mycobacterium tuberculosis* (Buetow et al., 2007). *Arabidopsis* mutants *ispF-1*, which had a null mutation in the *ispF* gene were albino lethal. In addition, chloroplasts of *ispF-1* were filled with vesicles rather than thylakoids (Hsieh and Goodman, 2006). The previous experimental results showed that MECS participated in the control of isoprenoid accumulation in plants. It also was demonstrated that MECS gene expression correlated with monoterpene indole alkaloid (MIA) accumulation (veau et al., 2000). Furthermore, the co-localization of MECS with a key enzyme geraniol 10-hydroxylase (G10H) indicated its indispensable role in the biosynthesis of TIAs (Burlat et al., 2004). HDS catalyzes the conversion of 2-C-methyl-D-erythritol-2,4-cyclodiphosphate into 1-hydroxy-2-methyl-2-(E)-butenyl-4-diphosphate, which is the penultimate enzymatic step of MEP pathway. The previous study has demonstrated that HDS (also named GCPE) was essentially involved in the MEP pathway in *E. coli* (Campos et al., 2001). A defective *HDS* mutant of *Arabidopsis* clb4 showed the arrested chloroplast development at the proplastid stage which suggested that *HDS* involved in the early chloroplast development in *Arabidopsis* (Guttiérrez-Nava et al., 2004). Even though HDSs from plant have not been fully characterized, previous researches indicated that HDS participated in controlling metabolic flux (Rodríguez-Concepción et al., 2003) and defended mechanism of plants (Gil et al., 2005). All of above reports suggested that both MECS and HDS were indispensable for organisms and could serve as potential target enzymes for metabolic engineering of TIAs biosynthesis. Unfortunately, until now there have been no reports on the cloning of the *MECS* gene and *HDS* gene from *R. verticillata*. To our knowledge, some stimuli such as MeJA, ABA and UV have positive effect on accumulation of plant secondary metabolites including TIAs. Thus, it is worthwhile to investigate the expression levels of relevant genes in TIAs biosynthesis responding to different elicitors.

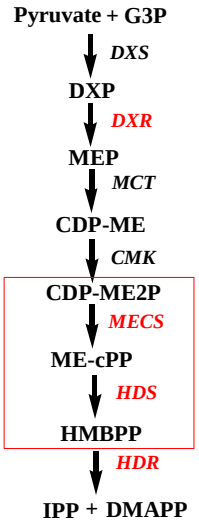

Fig 1. The methylerythritol phosphate pathway of isoprenoid biosynthesis DXS: 1-deoxy-D-xylulose5-phosphate synthase; DXR: 1-deoxy-D-xylulose5-phosphate reductoisomerase; MECT: 2-C-methyl-D-erythritol 4-phosphatecytidyl transferase; CMK: 4-(cytidine5'-diphospho)-2-C-methyl-D-erythritol4-phosphate kinase; MECS: 2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase; HDS: 1-hydroxy-2-methyl-2-(E)-butenyl4-diphosphate synthase. Enzymes labeled in red color represent those proteins whose corresponding cDNAs have been cloned from *R. verticillata*.

In the present study, a new *MECS* gene and *HDS* gene from *R. verticillata* were cloned, characterized by bioinformatic analysis and the tissue expression profile analysis. Our above work will be helpful to map and regulate two important enzymatic steps involved in *R. verticillata* TIAs biosynthetic pathway at the level of molecular genetics in the future.

Materials and methods

Plant materials and treatments

R. verticillata plant was cultivated in the plant garden of Southwest University (Chongqing, China). The roots, stems, leaves, fruits and flowers of *R. verticillata* were collected *in* September. After collection, the materials were immediately immersed into liquid nitrogen for total RNA isolation later. Total RNAs were isolated using the RNAplant reagent (Tiangen, China) according to the manufacturer's instructions and stored in -80 °C. The cell cultures of *R. verticillata* initiated from leaves were maintained on solid MS medium supplemented with 0.5 mg•L^{-1} 6-BA and 5 mg•L^{-1} NAA at 25 °C in darkness and sub-cultured every 4 weeks. In this study, for investigating the changes of *MECS* and *HDS* expression pattern according to various elicitors, *R. verticillata* cell cultures were respectively treated with 100 μM MeJA, 100 mg•L^{-1} ASA, 50 μM ABA and exposure to UV light. In contrast, cell cultures without any treatments served as control. Cell cultures collected after 24 hours were used for analysis of *RvMECS* and *RvHDS* expression profiles by quantitative PCR.

Cloning of the full-length cDNA of RvMECS and RvHDS

Single-strand cDNAs were synthesized from 5 μg of total RNA with an oligo (dT)17 primer (Table 1) and reversely transcribed according to the manufacturer's protocol (PowerScriptTM, Clontech, USA). After RNase H treatment, the single-strand cDNA mixtures were used as templates for PCR amplification of the conserved region of *MECS* and *HDS* from *R. verticillata*. Two pairs of degenerate primers, dfmecs and drmecs, dfhds and drhds (Table 1) were designed according to the conserved sequences of other plant *MECS* and *HDS* genes for amplifying the core cDNA fragment of *RvMECS* and *RvHDS* by standard gradient PCR amplification (from 55 to 68 °C) on BioRad My Cycler (USA). The core fragment of each gene was amplified and subcloned into pMD19-T vector (Takara, Japan) then transformed into *E. coli* strain DH5a for sequencing. The RACE-Ready cDNA for 3`-RACE was acquired by the method supplied by RNA PCR Kit (AMV) Ver.3.0 (TaKaRa). For the first PCR amplification of 3`-RACE cDNA, Rv*MECS*3-1 (Rv*HDS*3-1) and M13 Primer M4 were used as the primers (Table 1) and the 3`-RACE-ready cDNAs were used as templates. Then the 50-fold diluted first PCR products were used as the templates for the nested amplification of 3`-RACE, with Rv*MECS*3-2 (Rv*HDS*3-2) and M13 Primer M4 as the primers (Table 1). The PCR was conducted as the following procedures: 2 min at 94 °C, followed by 32 cycles of 30 sec at 94 °C, 30 sec at 52 °C and 1 min at 72 °C, then 8 min of extension at 72 °C.5`-RACE-ready cDNA samples of *R. verticillata* was prepared using SMARTTM RACE cDNA Amplification Kit (Clontech, USA) according to the manufacturer's protocol and used as templates for 5`-RACE. Advantage™ 2 PCR Kit (Clontech, USA) was used for amplifying 5'-ends of *RvMECS* and *RvHDS* cDNA. For the first PCR amplification of 5`RACE, Rv*MECS*5-1 (Rv*HDS*5-1)and Universal Primer A Mix (UPM, provided by Clontech) were used as the primers (Table 1), using 5`-RACE-ready cDNAs as templates. For the nested PCR amplification of 5`RACE, Rv*MECS*5-2 (Rv*HDS*5-2) and Nested Universal Primer A (NUP, provided by Clontech) were used as the primers (Table 1), and the 50-fold diluted products of the first PCR amplification were used as templates. PCR procedures of 5'-RACE amplifications were carried out under the following conditions: 25 cycles of amplification (30 sec at 94 °C, 30 sec at 68 °C, 3 min at 72 °C). By 3`-RACE and 5`-RACE, both ends of two cDNAs were obtained respectively and confirmed by sequencing. Contig Express (Vector NTI Suite 8.0) was used for assembling the sequences of 3`RACE, 5`-RACE and the core fragment, then the full-length cDNA sequence of *RvMECS* (*RvHDS*) were deduced, according to which two gene-specific primers: ffrvmecs and frrvmecs (ffrvhds and frrvhds) (Table 1) were designed to amplify the full-length of *RvMECS* (*RvHDS*) from 5`-RACE-ready cDNA samples.

Comparative and Bioinformatic Analysis

Comparative and bioinformatic analysis of *RvMECS* (*RvHDS*) were carried out online at the websites (http://www.ncbi.nlm.nih.gov and http://www.expasy.org). The sequence comparison was conducted through database search using BLAST program (Altschul et al., 1997). The subcellular location of *Rv*MECS (*Rv*HDS) was predicted by Target P (Nielsen et al., 2000). The multiple alignments of *Rv*MECS (*Rv*HDS) and MECSs (HDSs) from other species were aligned with CLUSTAL X (Thompson et al., 1997). A phylogenetic tree of each gene was constructed using MEGA 3.0 (Kumar et al., 2004) from CLUSTAL X alignments, based on the neighbor-joining method (Saitou and Nei, 1987).

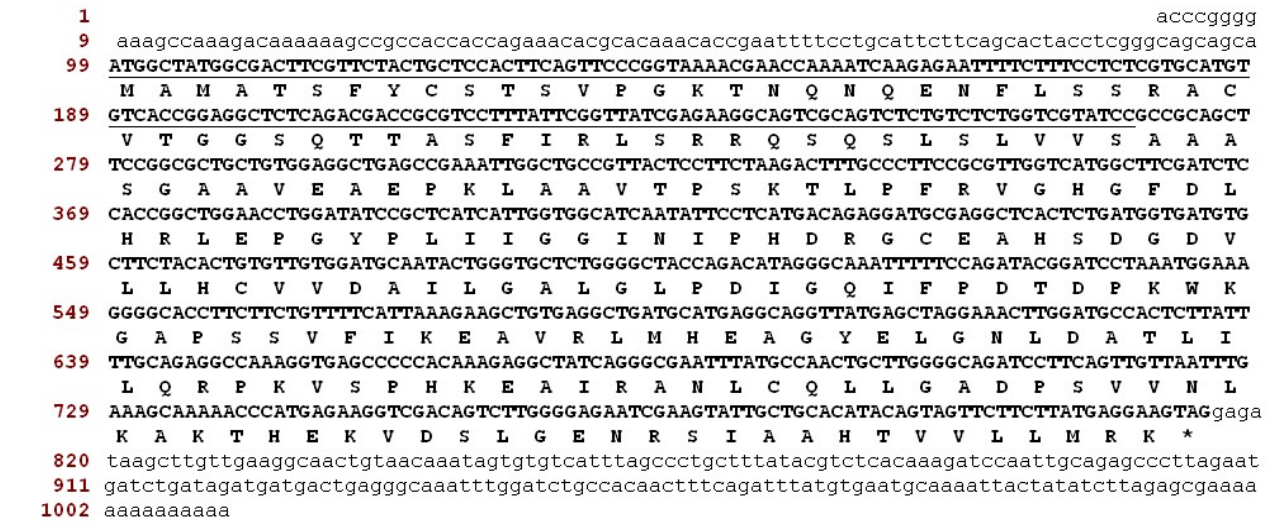

Fig 2A. The full-length cDNA sequence and the deduced amino acid sequence of *RvMECS*. The coding sequence and its deduced amino acid sequence were shown in capital letters, and the UTR were shown in small letters. The stop codon (TAG) was marked with an aster, the plastidial transit peptide was underlined.

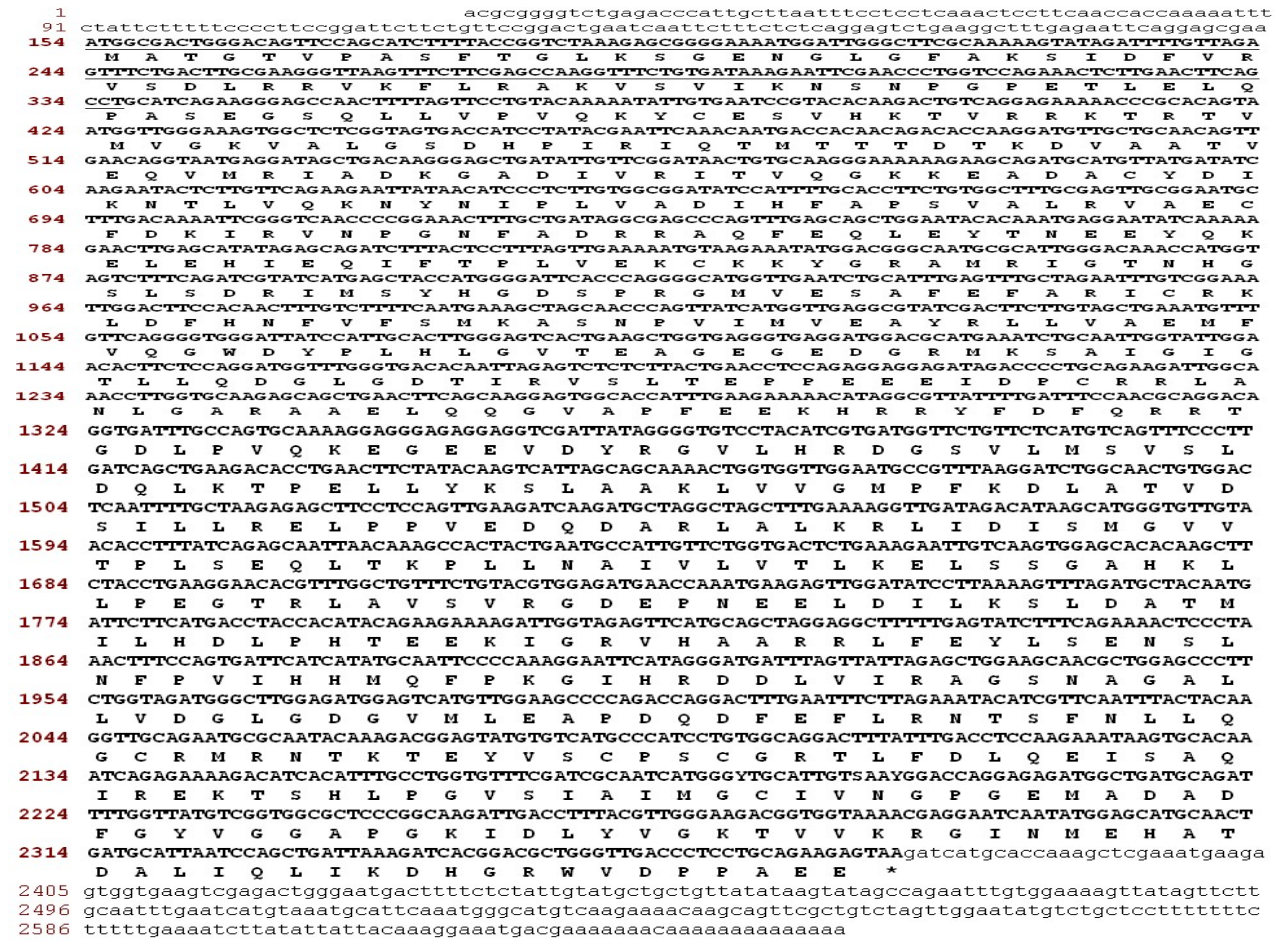

Fig 2B. The full-length cDNA sequence and the deduced amino acid sequence of *RvHDS*. The coding sequence and its deduced amino acid sequence were shown in capital letters, and the UTR were shown in small letters. The stop codon (TAG) was marked with an aster, the plastidial transit peptide was underlined.

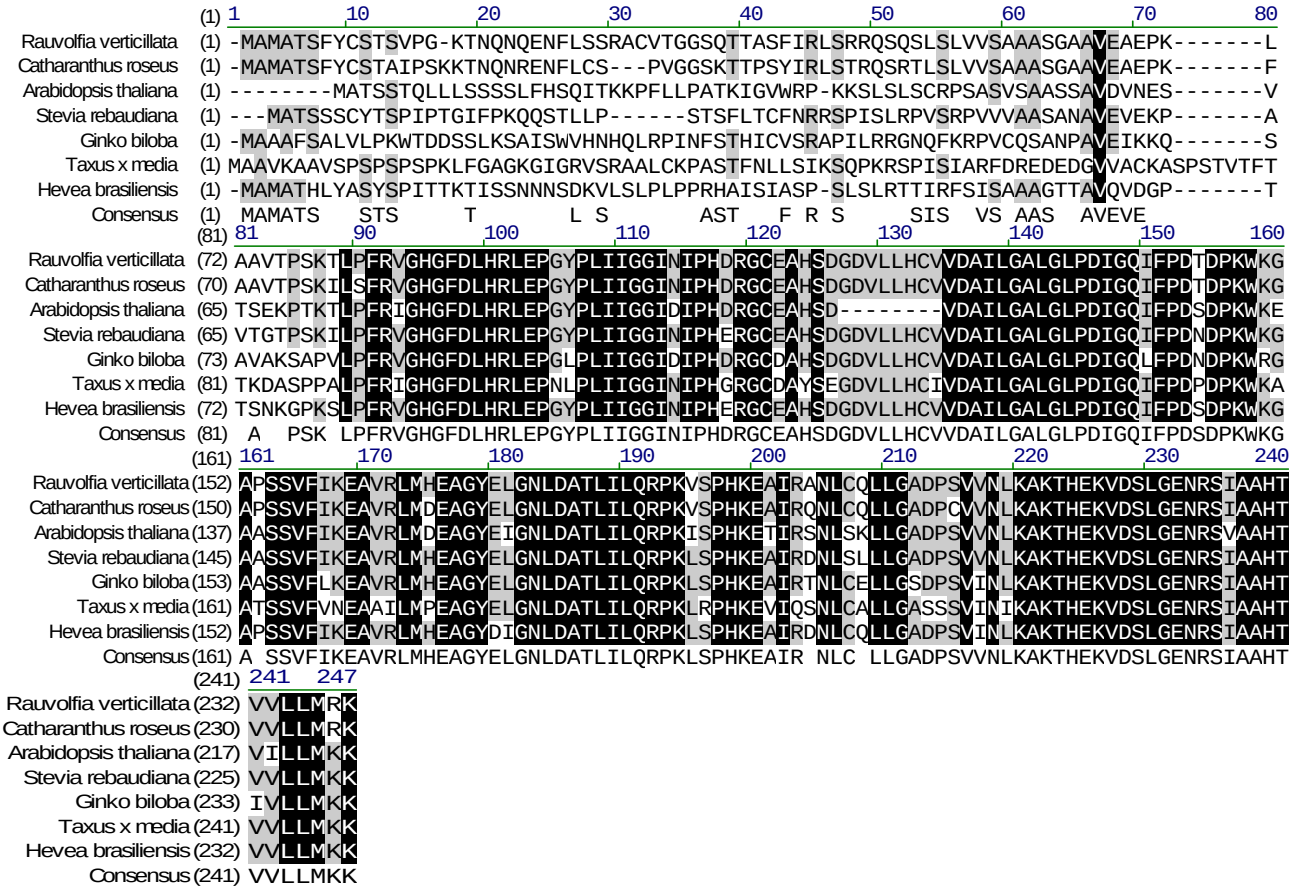

Fig 3A. Multi-alignment of amino acid sequences of *Rv*MECS with other MECSs. The identical amino acids were showed in white with black background and the conserved amino acids were showed in black with gray background, other amino acids were showed in black with white background.

Expression Profile Analysis of RvMECS and RvHDS

Quantitative RT-PCR was carried out to investigate the expression profile of *RvMECS* (*RvHDS*) in different tissues including roots, stems, leaves, flowers and fruits of *R. verticillata.* Changes of expression pattern under different elicitor treatments including 100 μM MeJA, 100 mg•L^{-1} ASA, 50 μM ABA and UV were also investigated. cDNA of each sample was synthesized using AMV Reverse Transcriptase (Takara, Japan) according to the manufacturer's instructions. Quantitative PCR of each gene was conducted with two primers: fexRvMECS and rexRvMECS (fexRvHDS and rexRvHDS) (Table 1) specific to the coding sequence of *RvMECS* (*RvHDS*) using SYBR Premix ExTaq (Takara, Japan). Amplifications were performed under the following conditions: 94 °C for 2 min, 40 cycles of denaturation at 94 °C for 15 s, annealing at 55 °C for 15 s and followed by extension at 68 °C for 20 s. Meanwhile, the house-keeping gene 18S rRNA gene was used as the reference gene.

Results and discussion

Cloning of the full-length cDNAs of RvMECS and RvHDS

Based on the conserved fragment of other plant *MECSs* (*HDSs*), two degenerate primers dfmecs and drmecs (dfhds and drhds) were designed for gradient PCR amplification of the core cDNA fragment of *MECS* (*HDS*) from *R. verticillata.* An approximate 400-bp product of *MECS* and a 820-bp product of *HDS* were respectively obtained and sequenced. The BLAST search revealed that core fragment of *RvMECS (RvHDS)* had high homologous with *MECS* (*HDSs*) genes from plant species. Especially, the similarity was extremely high when compared to *MECS* (*HDS*) from *Catharanthus roseus*. These results strongly suggested that the core fragment of *RvMECS* (*RvHDS*) had been obtained. By nested 3'-RACE and 5'-RACE, the 490 bp 3'-end and 660 bp 5'-end of *RvMECS* (the 572 bp 3'-end and 1493 bp 5'-end of *RvHDS*) were respectively obtained. By aligning and assembling ana-

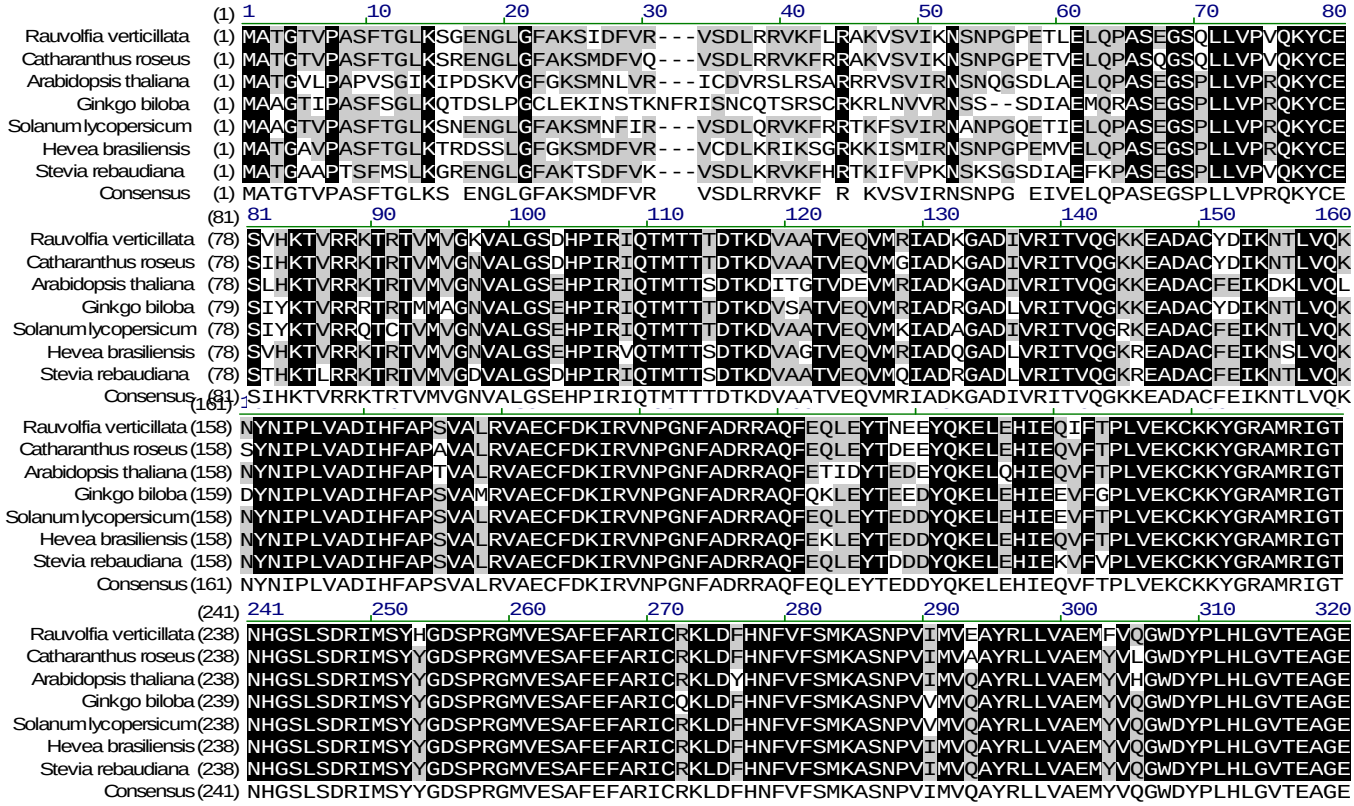

Fig 3B. Multi-alignment of amino acid sequences of *Rv*HDS with other HDSs. The identical amino acids were showed in white with black background and the conserved amino acids were showed in black with gray background, other amino acids were showed in black with white background.

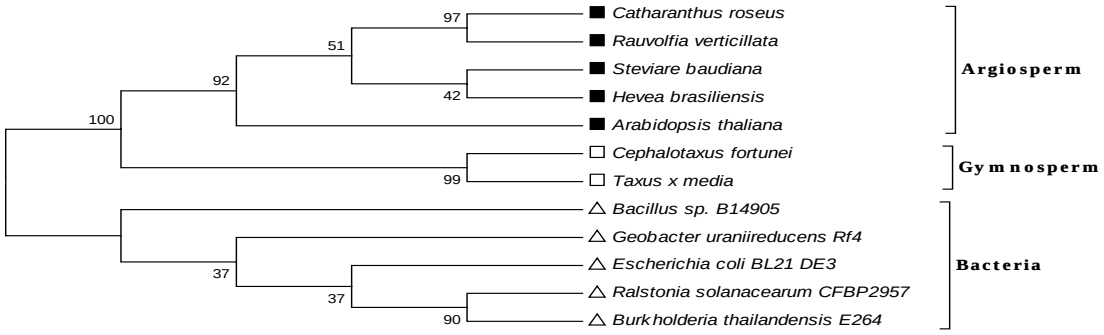

Figure 4A. Phylogenetic trees of MECSs from different organisms constructed by neighbor-joining method on MEGA 3. Bacteria-derived MECSs were marked with △, gymnosperms-derived MECSs were marked with □, argiosperms-derived MECSs were marked with■. The GenBank Accession Numbers: *Catharanthus roseus* AAF65155.1; *Stevia rebaudiana* ABG23395.1; *Cephalotaxus fortunei* ABD73009.1; *Taxus x media* ABB88956.1; *Arabidopsis thaliana* NP_850971.1; *Hevea brasiliensis* AAS94122.1; *Ralstonia solanacearum* YP_003745559.1; *Burkholderia thailandensis* E264 YP_442614.1; *Geobacter uraniumreducens* Rf4 YP_001232880.1; *Bacillus sp.* ZP_01725567.1; *Escherichia coli* CAQ33078.1.

lysis carried on Contig Express (Vector NTI Suite 8.0), the 1021-bp full-length cDNA sequence of *RvMECS* (the 2645-bp full length cDNA sequence of *RvHDS*) was deduced. Subsequently, the physical full-length *RvMECS* (*RvHDS*) cDNA was amplified and confirmed by sequencing (Figure 2). The ORF Finder program analysis on NCBI showed that the *RvMECS* had a 121-bp 5'untranslated region (UTR), a 196-bp 3'UTR and a 717-bp coding sequence which encoded a protein of 238-amino acid with a calculated molecular mass of 25.4 kDa and an isoelectric point of 8.32. *RvHDS* was predicted to had a 153-bp 5'UTR, a 272-bp 3'UTR and a 2220-bp coding sequence which encoded a protein of 740-bp amino acid with a calculated molecular mass of 82.0 kDa and an isoelectric point of 6.28. All above dates showed that a new *MECS* gene and a new *HDS* gene involved in upstream pathway of TIAs biosynthesis have been cloned from *R. verticillata*.

Comparative and bioinformatic analysis of RvMECS and RvHDS

BLAST research of amino acid sequences between *Rv*MECS (*Rv*HDS) and other MECSs (HDS*s*) were respectively conducted (Figure 3). The similarities between *Rv*MECS and other MECS*s* were*: C. roseus* (90.4% identities), *Stevia rebaudiana* (73.9% identities), *Ginkgo biloba* (63.2% identities) and *Arabiodopsis thaliana* (63.2% identities). It indicated that MECSs from different plant species varied a lot

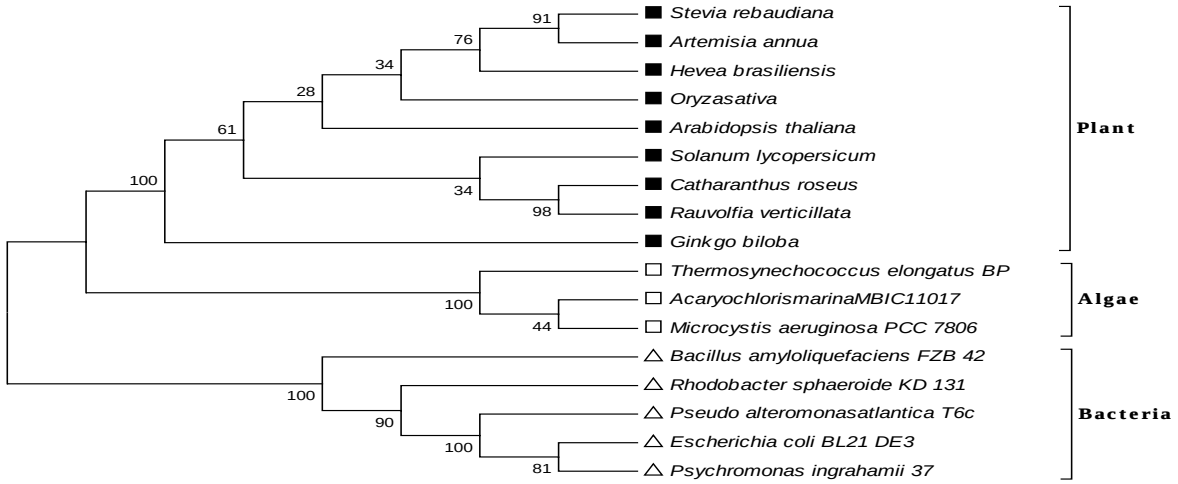

Fig 4B. Phylogenetic trees of HDSs from different organisms constructed by neighbor-joining method on MEGA 3. Bacteria-derived HDSs were marked with ○, algaes-derived HDSs were marked with □, plants-derived HDSs were marked with ■. The GenBank Accession Numbers: *Catharanthus roseus* AAO24774.1; *Stevia rebaudiana* ABG75916.2; *Ginkgo biloba* ABB78087.1; *Artemisia annua* ACT64770.1; *Arabidopsis thaliana* AAO15446.1; *Solanum lycopersicum* AAO15447.1; *Hevea brasiliensis* BAF98296.1; *Oryza sativa* EEE57327.1; *Escherichia coli* CAQ32886.1; *Microcystis aeruginosa* CAO90324.1; *Bacillus amyloliquefaciens* ABS74698.1; *Psychromonas ingrahamii* ABM03004.1; *Thermosynechococcus elongatus* NP_681786.1; *Pseudoalteromonas atlantica* YP_662686.1; *Rhodobacter sphaeroides* YP_002525666.1; *Acaryochloris marina* YP_001514549.1.

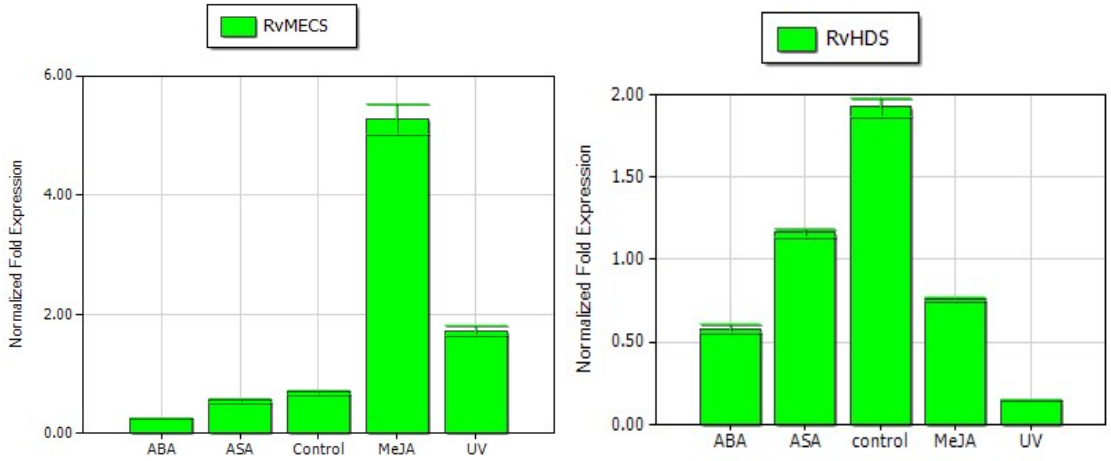

Fig 5. Expression profile of *RvMECS* and *RvHDS* under induction by elicitors including UV, 100 μM MeJA, 50 μM ABA and 100 $mg \cdot L^{-1}$ ASA. Total RNA samples were isolated from callus treated with UV, MeJA, ABA, ASA and without treatment (as the control), respectively, and analyzed by one-step RT-PCR.

in N-amino acids, which was non-catalytic region. However, amino acid sequences of plant MECSs were very consensus in catalytic region. Active site residues D8 (asparticacid), H10 (histidine), H42 (Zn^{2+} ligand) and E135 (glutamicacid, the binding site of Mg^{2+} and Mn^{2+}) of *E. coli* were also present in RvMECS and MECSs from other plant species. The BLAST result of amino acid sequence of *Rv*HDS was: *C. roseus* (94.7% identities), *Solanum lycopersicum* (89.1% identities), *A. thaliana* (84.6% identities), *G. biloba* (82.8% identities). Target P was used for predication of sub-locations of *Rv*MECS and *Rv*HDS. *Rv*MECS protein was predicted to be localized in plastid with a 57-amino acid plastid targeting sequence at its N-terminal end. *Rv*HDS protein was also predicated to obsess a 61-amino acid signaling target sequence. The predications were consistent with the fact that *MECS* and *HDS* were two genes involved in MEP pathway which located in plastid (Lichtenthaler et al., 1997). The previous study of MECS and HDS from *G. biloba* demonstrated the localization of MECS and HDS was plastid (Kim et al., 2006; Kim and Kim, 2010). Additionally, secologanin, the direct precursor of TIAs biosynthesis was also located in plastid (Ymazaki et al., 2003). Using MEGA 3.0 based on CLUSTAL X alignments, a phylogenetic tree of MECSs (HDSs) was respectively constructed from different organisms (Figure 4). The result demonstrated that MECSs could be divided into three groups, those of angiosperm, gymnosperm and bacteria. *Rv*MECS was shown to belong to angiosperm category. HDSs were shown to derive from an ancestor gene and evolved into three groups including plants, algaes and bacteria HDS group. *Rv*HDS had higher identity with plant HDSs than bacterium and algae HDSs. All the analysis results strongly suggested that *Rv*MECS and *Rv*HDS

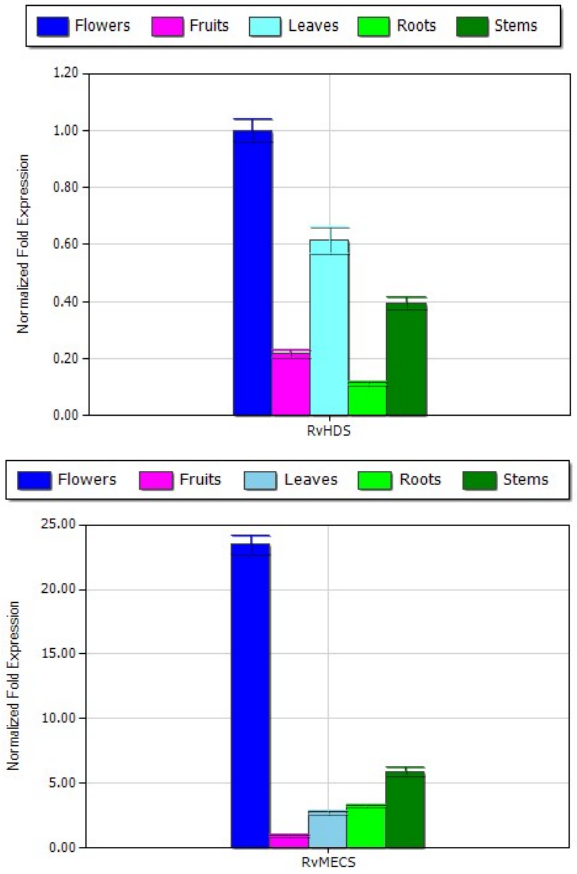

Fig 6. Expression profile of *RvMECS* and *RvHDS* in different tissues of *R. verticillata*. Total RNA samples were isolated from roots, stems, leaves, flowers and fruits respectively, and subjected to quantitative RT-PCR analysis.

we obtained were two plant proteins involved in the mevalonate-independent biosynthesis.

Expression profile analysis

Quantitative RT-PCR was used for analysis of expression profile of *RvMECS* (*RvHDS*) in different tissues with house-keeping gene (18S rRNA gene) as a reference gene. The result showed *RvMECS* expression could be detected in all tissues, suggesting that *RvMECS* was constitutively expressed in different organs but at different levels. For instance, the highest expression level of *RvMECS* was found in flowers of *R. verticillata*, followed by leaves, stems, fruits and roots (Figure 5). The expression level of *RvHDS* was also detected in all tissues, with highest amount in flowers followed by leaves, stems, fruits and roots (Figure 5).To investigate the effects of elicitor treatments on expression levels Rv*MECS* and *RvHDS*, quantitative PCR was carried out to monitor the expression changes of two genes upon treatments including 100 μM MeJA, 100 mg•L^{-1} ASA, 50 μM ABA and UV. The result showed that the expression level of *RvMECS* was both strongly increased by MeJA and UV treatments, among which the highest transcript level of *RvMECS* was found induced by MeJA treatment. However, ABA and ASA showed had no positive effect on *RvMECS* expression (Figure 6). The result suggested that *RvMECS* was a highly-regulated gene for basic physiological and biochemical processes in *R. verticillata*. Different from *Rv*MECS, *Rv*HDS could not be up-regulated by four elicitors, insteadly, the expression of *Rv*HDS was greatly inhibited by elicitors including ABA, ASA, MeJA and UV (Figure 6). The result indicated that the regulation mechanism of *Rv*HDS was different from that of *Rv*MECS.

Conclusions

MECS and HDS catalyze the fifth and the sixth reaction of the MEP pathway which produces precursors for TIAs biosynthsis (Altincicek et al., 2002), then they are deemed as two ideal targets for metabolic engineering of the isoprenoid biosynthetic pathway. In the present study, we have successfully isolated and characterized the *RvMECS* cDNA and *RvHDS* cDNA from *R. verticillata* for the first time. Our present study also indicated that MeJA and UV could up-regulate the expression of *RvMECS*. However, elicitors including UV, MeJA, ABA and ASA had no positive effects on the expression profile of *RvHDS*. The results provide direct evidence that *RvMECS* is an elicitor-responsive gene and can be effectively up-regulated at least at the transcription level by certain kinds of elicitors. The present results imply the possibility of improving TIAs production through the up-regulation of enzymatic genes after induction of elicitors as well. Cloning and characterization analysis of *RvMECS* and *RvHDS* will facilitate the understanding of the biosynthesis of TIAs including reserpine and ajmalicine. It has been demonstrated in previous reports that overexpressing key enzymatic genes is a promising stategy to enhance the accumulation of medicinal alkaloids (Yang et al., 2011). Based on the present research, plant expression vector containing the *RvMECS* and *RvHDS* can be constructed. In combination with the establishment of genetic transformation system, potential roles of *RvMECS* and *RvHDS* in improving TIAs production by genetic engineering can be tested in the near future.

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