Full Length Research Paper

The HDR gene involved in the TIA pathway from *Rauvolfia verticillata*: Cloning, characterization and functional identification

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1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase (HDR, EC 1.17.1.2) catalyzes the last reaction of the methylerythritol phosphate (MEP) pathway. The full-length cDNA sequence of HDR was cloned and characterized from terpenoid-indole-alkaloid-producing Rauvolfia verticillata. The new cDNA was named as RvHDR and submitted to GenBank® to be assigned with an accession number: EU034699. The full-length cDNA of RvHDR was 1679-bp containing a 1389-bp open reading frame (ORF) encoding a polypeptide of 462-amino acids with a calculated molecular mass of 52 kDa and an isoelectric point of 5.26. Comparative and bioinformatic analyses revealed that *RvHDR* had extensive homology with HDRs from other plant species and contained a conserved transit peptide for plastids. The phylogenetic indicated that all HDRs could be divided into three groups and RvHDR belonged to plant HDRs family. RvHDR was found to be expressed in all tested tissues including roots, stems, leaves, flowers and fruits but at different levels. The highest expression level was found in flowers, and higher expression level in leaves and fruits; the expression level was low in roots and lowest in stems. Expression profiling analyses revealed that RvHDR expression was induced by exogenous elicitors including methyl jasmonate, acetyl salicylic acid, abscisic acid and UV, and showed the transcription levels were all up-regulated compared to the control. Finally, RvHDR was transformed into the E. coli HDR mutant strain MG1655 ara<>HDR, which was able to rescue the lethal phenotype of the *E. coli* HDR mutant. This confirmed that RvHDR had the typically function of HDR gene. The cloning, characterization and functional identification of RvHDR will be helpful to understand more about the function of HDR at the level of molecular genetics and help to unveil the biosynthetic mechanism of TIAs precursor and provides a candidate gene for metabolic engineering of the TIAs pathway in R. verticillata.

Key words: Rauvolfia verticillata, HDR gene, cloning, expression profile, functional complementation.

INTRODUCTION

Terpenoid Indole Alkaloids (TIAs), which constitute one of

the largest groups of natural products, provide many pharmacologically active compounds. *Rauvolfia alkaloids*, such as ajmalicine and reserpine are therapeutically applied for hypertension and cardiac disorders because of their antihypertensive and antiarrythmic properties (Anitha et al., 2006). *Rauvolfia verticillata* is a rare

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medicinal shrub belonging to the family *Apocynaceae*, which is the main source of reserpine and ajmalicine in China (Li et al., 1962). In pharmaceutical industries, reserpine is in great demand. Even though the chemical synthesis of reserpine is possible, it costs more than extracting it from natural resources (Farooqi et al., 2001). So it is eager for finding an efficient way to provide 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 reserpine of TIAs belongs to isoprenoids is synthesized by condensation of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) units usually synthesized by the methylerythritol phosphate (MEP) pathway in plastids (Ramos-Valdivia et al., 1998).1-hydroxy-2-methyl-2-(E)-butenyl 4-diphosphate reductase (HDR) simultaneously synthesizes IPP and DMAPP in the last step of the pathway, which is noted a key enzyme in the precursors biosynthesis of isoprenoids. Overexpression studies using sequences from cyanobacteria (Synechocystis) and plants (Adonis aestivalis) showed that the activity of the HDR enzyme was limiting for isoprenoid biosynthesis in E. coli (Cunningham et al., 2000). Studies on overexpression of tomato HDR cDNA in Arabidopsis plants led to the conclusion that plant HDR protein plays a key role in controlling the biosynthesis of plastid isoprenoids (Botella-Pavia et al., 2004). In Arabidopsis thaliana, HDR was able to rescue the lethal phenotype of an E. coli HDR mutant, and plants with loss-of-function in the Arabidopsis HDR gene are albino (Guevara et al., 2005; Hsieh et al., 2005). All these reports suggested that HDR may be an ideal target enzyme for metabolic engineering of reserpine biosynthesis. Unfortunately, until now there have been no reports on the cloning of the HDR gene from R. verticillata. In the present study, a new HDR gene from R. verticillata was cloned, characterized by bioinformatic analysis and the tissue expression profile analysis, and then finally functionally expressed in Escherichia coli, which will enable us to map and regulate an important step 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 cultured in the plant garden of Southwest University (Chongqing, China). The roots, stems, leaves, fruits and flowers were collected from *R. verticillata in* September. After collection, the materials were immediately immersed into liquid nitrogen to store for total RNA isolation. Total RNAs were isolated separately using the RNAplant reagent (Tiangen, China) according to the manufacturer's instructions. After isolation, total RNAs was stored in -80 °C for future uses. The cell cultures, initiated from young leaves of *R. verticillata*, were maintained on solid MS medium supplemented with 0.5 mg.L⁻¹ 6-benzyl aminopuine (6-BA)

5 mg.L⁻¹ α-Naphthalene acetic acid (NAA) at 25 °C in darkness and subcultured every 4 weeks. In this study for investigating induction by various elicitors, *R. verticillata* cell cultures were respectively dipped into the appropriate treatments such as 100 μM methyl jasmonate (MeJA), 100 mg.L⁻¹ acetyl salicylic acid (ASA), 50 μM abscisic acid (ABA) and exposed under UV light, using cell cultures without any treatments as control. Cell cultures were collected after 24 h treatment for analyses of *RvHDR* expression profiles by semiquantitative one-step RT-PCR.

Cloning of the full-length cDNA of RvHDR

Single-strand cDNAs were synthesized from 5 μ g of total RNA with an oligo(dT)17 primer 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 *HDR* from *R. verticillata.* Two degenerate primers, dfhdr and drhdr (Table 1), were designed according to the conserved sequences of other plant *HDR* genes and used for the amplification of the core cDNA fragment of *RvHDR* by standard gradient PCR amplification (from 55 – 68°C) on BioRad My Cycler (USA). The core fragment was amplified and subcloned into pGEM T-easy vector (Promega, USA), transformed into *E. coli* strain DH5a followed by sequencing. The core fragment was subsequently used to design the gene-specific primers for the cloning of the full-length cDNA of *RvHDR* by the technology of rapid amplification of cDNA ends (RACE).

SMARTTM RACE cDNA Amplification Kit (Clontech, USA) was used to clone the 3'-end and 5'-end of *RvHDR* cDNA. The first strand 3'-RACE-ready and 5'-RACE-ready cDNA samples from *R. verticillata* were prepared according to the manufacturer's protocol (SMARTTM RACE cDNA Amplification Kit, User Manual, Clontech, USA) and used as templates for 3'-RACE and 5'-RACE, respectively.

Two 3'-gene-specific primers and the universal primers provided by the kit were used to amplify the 3'-end of RvHDR. For the first PCR amplification of 3'-RACE of RvHDR cDNA, RvHDR3-1 and Universal Primer A Mix (UPM, provided by Clontech) were used as the primers and the 3'-RACE-ready cDNAs were used as templates .Then the first PCR products were used as the templates for the nested amplification of 3'-RACE, RvHDR3-2 and Nested Universal Primer (NUP, provided by Clontech) were used as the second PCR amplification. The 5'-end of RvHDR cDNA was amplified using two 5'-gene-specific primers and the universal primers (UPM and NUP) provided by the kit. For the first PCR amplification of 5 RACE, RvHDR5-1 and UPM were used as the first PCR primers, and 5'-RACE-ready cDNAs were used as templates. For the nested PCR amplification of 5'RACE, RvHDR5-2 and NUP were used as the nested PCR primers, and the products of the first PCR amplification were used as templates. For the first and nested PCR amplification of RvHDR cDNA 3' and 5'-ends, Advantage™ 2 PCR Kit (Clontech, USA) was used. The first and nested PCR procedures were carried out at the same conditions described in the protocol (SMARTTM RACE cDNA Amplification Kit, User Manual, Clontech): 25 cycles of amplification (30 sec at 94 °C, 30 s at 68 °C, 3 min at 72 °C). By 3'-RACE and 5'-RACE, both ends of RvHDR were respectively obtained.

By assembling the sequences of 3`RACE, 5`-RACEand the core fragment on Contig Express (Vector NTI Suite 8.0), the full-length cDNA sequence of *RvHDR* was deduced. According to the deduced *RvHDR* cDNA sequence, two gene-specific primers: ffrvhdr and frrvhdr were used to amplified the full-length of *RvHDR* from 5`-RACE-ready cDNA samples through proof-reading PCR. All the PCR amplificons were subcloned into pGEM-T vector and followed by sequencing. Finally *RvHDR* was submitted to GenBank to be assigned with an accession number.

Primers	Orientation	Sequence
dfhdr	Sense	5`-GT(C/T)GAGCG(C/T)GC(A/T/C)GT(G/T/C)CAGAT(G/T)GC-3`
drhdr	Antisense	5`-CATC(C/T)TC(A/C)AC(G/A/T)(A/G)CCTT(A/G)TC(A/G/C/T)GG-3`
RvHDR3-1	Sense	5`-GGGTTTCGATCCAGATAATGATC-3`
RvHDR3-2	Sense	5`-GGACGATGATGCGTAAGTATG-3`
RvHDR5-1	Antisense	5`-GCCGCTCTTGAGTTGCATCACAGA-3`
RvHDR5-2	Antisense	5`-GACCTCCAAGGATATAGTCGCAAAC-3`
ffrvhdr	Sense	5`-ACGCGGGGACCTCAAAAAGAAG-3`
frrvhdr	Antisense	5`-ACGAAAAGAGCTATTTATACATCAG-3`
fexRvHDR	Sense	5`-ATGGCTATCTCTCTGCAATTCTCC-3`
rexRvHDR	Antisense	5`-CTATGCCAGTTGCAGGGCTTC-3`
18SF	Sense	5`-ATGATAACT CGACGGATCGC-3`
18SR	Antisense	5`-CTTGGATGTGGTAGCCGTTT-3`
F-cdsRvHDR	Sense	5'-CCGGATCCGCTATCTCTCTGCAATTCTC-3'
R-cdsRvHDR	Antisense	5'-CCAAGCTTCTATGCCAGTTGCAGGGCTT-3'

Table 1. The nucleotide sequences of oligonucleotide primers.

Comparative and bioinformatic analysis

Comparative and bioinformatic analyses of *RvHDR* 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 *RvHDR* was predicted by TargetP (Emanuelsson et al., 2000). The multiple alignments of *RvHDR* and HDRs from other species were aligned with CLUSTALX (Thompson et al., 1997). A phylogenetic tree was constructed using MEGA 3.0 (Kumar et al., 2004).from CLUSTAL X alignments. The neighborjoining method (Saitou et al., 1987) was used to construct the phylogenetic tree.

Expression profile analyses of RvHDR

Semi-guantitative one-step RT-PCR was carried out to investigate the expression profile of *RvHDR* in different tissues including roots, stems, leaves, flowers and fruits of R. verticillata and under different elicitor treatments including 100 µM MeJA, 100 mg.L⁻¹ ASA, 50 µM ABA and UV, respectively. Aliquots of 0.4 µg total RNAs extracted from each sample of R. verticillata were used as templates in onestep RT-PCR reaction with two primers: fexRvHDR and rexRvHDR specific to the coding sequence of RvHDR using one-step RNA PCR kit (TaKaRa, Japan). Amplifications were performed in a volume of 25 µL under the following conditions: 50 °C for 30 min, 94°C for 2 min followed by 25 cycles of amplification (94°C for 30 s, 58 ℃ for 30 s, 72 ℃ for 2 min). Meanwhile, the RT-PCR reaction for the house-keeping gene (18S rRNA gene) using specific primers 18SF and 18SR designed according to the conserved regions of plant 18S rRNA gene was performed to estimate whether equal control. PCR products (15 µL) were separated on 1.0% agarose gels stained with goldview.

Complementation of the E. coli HDR mutant

The *E. coli* HDR mutant strain MG1655 ara<>HDR (HDR, namely lspH) was maintained on Luria-Bertani (LB) medium containing 50 mg.L⁻¹ kanamycin (Kan) and 0.2% (w/v) arabinose (Ara) (McAteer et al., 2001), but not able to form colonies on LB medium containing

0.2% (w/v) glucose (Glc) in the absence of Ara. According to the complementation strategy the E. coli HDR mutant strain MG1655 ara<>HDR was used to test the biological function of *RvHDR* in this experiment. The coding region of RvHDR was amplified by PCR using primers F-cds RvHDR and R-cdsRvHDR. Both of the fragment of RvHDR and the plasmid pQE30 were digested with BamH I and Hind III for 10 h. Subsequently, the coding region of RvHDR was cloned into the expression vector pQE30 to obtain the plasmid pQE30-RvHDR. The pQE30-RvHDR was transformed into the E. coli HDR mutant strain and selected on LB plates containing 50 mg.L⁻¹ Kan, 50 mg.L⁻¹ ampicillin (Amp), 0.2% Glc, and 0.5 mM IPTG. The presence of the pQE30-RvHDR plasmid in surviving colonies was verified. As a control, the empty pQE30 vector was transformed into the E. coli HDR mutant and selected on LB plates containing 50 mg.L⁻¹ Kan, 50 mg.L⁻¹ Amp, and 0.2% Glc, and 0.5 mM IPTG. The transformants containing the pQE30 empty vector cannot grow on medium containing 0.2% Glc and 0.5 mM IPTG.

RESULTS

Cloning of the full-length cDNA of *RvHDR*

Based on the conserved fragment of other plant HDR sequences, such as Arabidopsis thaliana, Picrorhiza kurrooa, Vitis vinifera, Hevea brasiliensis and etc, two degenerate primers (dfhdr and drhdr) were designed and used for gradient PCR-amplification of the core cDNA fragment of HDR from R. verticillata. Following PCR amplification, an approximately 1000-bp product was obtained at 62.9℃ and sequenced. The BLAST search revealed that the 974-bp cDNA core fragment had high homologous with HDR genes from plant species such as Solanum tuberosum, Nicotiana tabacum, Pinus taeda and etc. These strongly suggested that the core fragment of *RvHDR* had been obtained. Thus, this fragment was used to design gene specific primers for both 5'-RACE and 3'-RACE. By nested 3'-RACE and 5'-RACE, the 588 bp 3'end and 929 bp 5'-end of *RvHDR* were respectively

1																				a	cgc	ggg	gac	ctc	aaa	aaq	yaag	gaa	aaga	aaa
	саа	gag	gtg	cgc	gct	tca	icac	aat	ttg	ctc	tcc	ata	acco	aga	cgg	làca	igtc	tcc	tgo	taa	cgt	gcg	сса	att	ccg	tt	cggc	gac	ttt	cac
32	ATG	GCT	ATC	TCI	CTC	CAA	TTC	TCC	CGGT	CTC	TCC	AC	CGC	ACG	GCG	GAC	CTC	GCC	TTC	CCG	GAG	CCG	AGA	ATC	TTC	CG	STGC	TGG	AAA	ССТ
	М	А	I	s	L	Q	F	S	G	L	s	т	R	т	А	D	L	A	L	P	Е	P	R	I	F	R	С	W	к	P
122	GTG	TCT	GTT	CGA	TGC	TCC	GCI	GCC	CGGG	GAA	GCI	CC	GCI	GTT	TCT	TCP	TCC	TCG	ACT	GAG	TCA	GAC	TTC	GAT	GCC	AA	GAAA	TTC	AGG	CAC
	v	s	v	R	С	s	A	A	G	Е	А	P	Α	v	s	s	s	s	т	Е	s	D	F	D	А	ĸ	к	F	R	н
212	AAC	TTG	ACT	AGA	AGC	AAG	AAT	'TAC	CAAT	CGG	AGA	GG'	TTT	GGA	CTC	AAA	GAA	GAG	AGC	ATG	GAG	CTG	ATG	AAC	CGC	GA	TAC	GCA	AGT	GAC
	N	L	т	R	s	ĸ	N	Y	N	R	R	G	F	G	L	ĸ	Е	Е	s	М	Е	L	М	N	R	Е	Y	A	s	D
302	ATC	ATA	CAA	AAC	TTG	AAG	GAC	AA	IGGA	TAT	GAA	TAC	CACA	TGG	GGA	AAC	GTC	ACT	GTC	:AAA	CTT	GCA	GAA	GCA	TAT	GG'	TTT	TGC	TGG	GGC
	I	I	Q	к	L	ĸ	D	N	G	Y	Е	Y	т	W	G	N	v	т	v	к	L	А	Е	А	Y	G	F	С	W	G
392	GTC	GAG	CGT	GCA	GTG	CAC	ATT	GC.	TAT	GAG	GCC	AGI	AAA	CAA	TTT	CC7	ACA	GAG	AGC	ATA	TGG	CTA	ACC	AAT	GAA	AT'	TTAT	CAC	AATO	CCT
	v	Е	R	А	v	Q	I	A	Y	Е	А	R	ĸ	Q	F	P	т	Е	R	I	W	L	т	N	Е	I	I	н	N	P
482	ACT	GTT	AAT	GAG	CGG	TTG	GAG	GA	ATG	AAG	GTA	AAG	GAA	ATC	CCC	CTI	GAT	GAT	GGG	GAG	AAA	CAA	TTT	GAT	GTT	GT	GAC	CAG	GGC	GAT
	т	v	N	Е	R	L	Е	Е	м	к	v	ĸ	Е	I	P	L	D	D	G	Е	к	Q	F	D	v	v	D	Q	G	D
572	GTT	GTA	ATT	TTG	CCI	GCI	TTT	'GG/	\GCT	GGT	GTC	GA	GAG	ATG	СТС	ACT	CTG	AGC	AAC	AAG	AAT	GTA	CAA	ATA	GTT	GA	CACC	ACT	TGC	CCA
	v	v	I	L	P	А	F	G	А	G	v	D	Е	м	L	т	L	s	N	к	N	v	Q	I	v	D	т	т	С	P
662	TGG	GTG	GTA	AAG	GTC	TGG	AAT	TC	IGTI	GAA	AAG	CA	AAG	AAG	GGI	GAT	TAT	ACA	TCA	ATT	ATC	CAT	GGI	AAA	TAT	TC	CAT	GAG	GAG	ACT
	W	v	v	К	v	W	N	s	v	Е	к	н	к	к	G	D	Y	т	s	I	I	н	G	к	Y	s	н	Е	Е	т
752	ATT	GCT	ACC	TCA	TCC	TTT	GCA	GG2	AAA	TAT	ATC	AT	GTG	AAG	AAC	ATG	AAA	GAG	GCI	ATA	TAT	GTT	TGC	GAC	TAT	AT(CTI	GGA	GGT	CAA
	I	А	т	s	s	F	А	G	к	Y	I	I	v	ĸ	N	М	к	Е	А	I	Y	v	С	D	Y	Ι	L	G	G	Q
842	CTA	GAT	GGA	TCT	AGC	TCA	ACC	AA	GAA	GCA	TTT	ATC	GAG	AAA	TTT	AAA	AAT	GCT	GTT	TCT	AAG	GGT	TTC	GAT	CCA	GA'	TAAT	GAT	CTC	$\mathbf{T}\mathbf{T}\mathbf{G}$
	L	D	G	s	s	s	т	K	Е	А	F	М	Е	к	F	ĸ	N	А	v	s	к	G	F	D	P	D	N	D	L	L
932	AAA	GTT	GGC	ATT	GCA	AAC	CAA	AC/	ACA	ATG	CTC	AAC	GGA	GAA	ACA	GAG	GAG	ATT	GGI	AAA	TTG	ATT	GAC	AGG	ACG	AT	SATC	CGT	AAG	PAT
	K	v	G	I	А	N	Q	т	т	м	L	к	G	Е	т	Е	Е	I	G	к	L	I	Ε	R	т	М	М	R	к	Y
1022	GGA	GTG	CAA	AAT	ATC	AAC	GAC	CAC	CTTT	ATG	AGT	TTC	CAAC	ACC	ATC	TGT	GAT	GCA	ACI	'CAA	GAG	CGG	CAA	GAT	GCC	AT(TAT	AAG	CTG	GTT
	G	v	Q	N	I	N	D	н	F	М	s	F	N	т	I	С	D	A	т	Q	Е	R	õ	D	А	М	Y	к	L	v
1112	GAT	CAA	TCT	GTA	GAT	CTT	ATG	CT	AGTA	ATT	GGA	GGG	TGG	AAC	TCO	AGC	AAC	ACT	TCO	CAT	CTA	CAA	GAG	ATC	GCT	GA	AGAA	CGT	GGA	ATT
	D	õ	s	v	D	L	м	L	v	I	G	G	W	N	s	s	N	т	s	н	L	õ	Е	I	А	Е	Е	R	G	I
1202	CCC	TCA	TAT	TGG	ATT	GAI	AGT	GAC	GAG	AGA	ATA	GG	CCI	GGA	AAC	AGA	ATA	AGT	TAC	AAG	CTC	CTG	CAI	GGT	GAG	TT	GTI	GAG	AAA	GAG
	P	s	Y	W	I	D	s	Е	Е	R	I	G	P	G	N	R	I	s	Y	к	L	L	Н	G	Е	L	v	Е	к	Е
1292	AAT	TTT	CTG	CCA	GAA	GGI	'CCC	ATC	CACA	ATA	GGA	GTZ	ACT	TCT	GGI	'GCC	TCA	ACA	CCC	GAT	AAG	GTT	GTI	GAA	GAT	GT	CTT	GTC	AAG	бта
	N	F	L	P	Е	G	P	I	т	I	G	v	т	s	G	А	s	т	P	D	к	v	v	Е	D	v	L	v	к	v
1382	TTT	GAC	ATC	AAA	CGC	GAA	GAA	GCC	CTG	CAA	CTC	GCI	ATAG	gtt	aaq	fact	gca	gtc	саа	aat	gtc	aat	gta	act	ata	gga	attg	ttc	caga	agc
	F	D	I	ĸ	R	Е	Е	А	L	Q	L	A	*																	
1472	taa	ttc	agc	gga	aca	gtc	gta	tto	catg	ata	gat	tct	caa	acc	aca	ago	ttg	gta	tat	gta	aca	aaa	act	gga	aat	gca	atto	tga	tgta	ata
1562	aat	agc	tct	ttt	cgt	aaa	laaa	laaa	aaaa	a																				

Figure 1. The full-length cDNA sequence and the deduced amino acid sequence of *RvHDR*. 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.

obtained. By aligning and assembling the sequences of 3'-RACE, 5'-RACE and the core fragment on Contig Express (Vector NTI Suite 8.0), the full-length cDNA sequence of RvHDR with 1679-bp was deduced. Finally the physical full-length RvHDR cDNA was amplified and confirmed by sequencing (Figure 1). The sequencing results showed that RvHDR had the 121-bp 5` untranslated region (UTR), the1389-bp coding sequence and the 169-bp 3` UTR including the polyA tail. Then, the full-length RvHDR sequence was submitted to GenBank and assigned an accession number: EU034699. The ORF Finder program analysis on NCBI showed that the RvHDR contained a 1389-bp ORF encoding a protein of 462-amino acid with a calculated molecular mass of 52 kDa and an isoelectric point of 5.26.All dates show that a new full length HDR gene involved in TIAs biosynthesis had been cloned.

Comparative and bioinformatic analysis of RvHDR

Sequence BLAST research showed that the deduced

amino acids of *RvHDR* from *R. verticillata* had high similarities with *HDRs* from other plant species, such as *Picrorhiza kurrooa* (80% identities), *Vitis vinifera* (81% identities), *Hevea brasiliensis* (79% identities) and *Adonis palaestina* (78% identities). Thus, the BLAST analysis results indicated that *RvHDR* belonged to the HDR family. To analyze the presence of peptide signal, Target P was used to predict the specific plastid targeted.

RvHDR protein was predicted to have plastid localization and had a 37-amino acid sequence with characteristics of plastidial targeting sequences at its Nterminal end. This was consistent with the fact that TIAs was synthesized in plastids (Yamazaki et al., 2003). Based on the multiple alignments, it was found that all aligned plant *HDRs* had a plastidial transit peptide at the N terminus, but the *E. coli* HDR protein lacked the Nterminal extension (Figure 2). This specific N-terminal extension comprised a signal sequence for plastid import, consistent with the subcellular localization of the MEP pathway in plants (Lichtenthaler et al., 1997). Furthermore, four conserved cysteine residues were found in *RvHDR* owned by all plant HDRs that might

	(1)	1 10	20	30	40	50	60
Adonis_palaestina	(1)	MA	ISLQFCRFST	PSDLSFPE-TR	SSTRLYRSKKP	FSVRCHSEGP	SGSSS-T
Hevea brasiliensis	(1)	MA	VSLOLCRVSL	RSDLFSRI	ENLAPLNRRKF	LSVRCAAGGD	ESSAGSV
Vitis vinifera	(1)	MAN	MSLOLCRFST	FSDRSLPE-AF	AGIGVFRRRKP	LSVRCSGESE	SSSSSSV
Artemisia annua	(1)	MA	ASLÕLTPLST	RTDYLSLP	ADIKVFRCRKP	LTVRCSGGDT	S
Stevia rebaudiana	(1)	MA	ATLRESPEST	CTELSLP	-DVKLFRCRKP	LSVRCSGGDS	SSPSV
Escherichia coli	(1)						
Ginkoo biloba	(1)	MAOACAVSGILA	ASHSOVKLDS	TYVSGLKM-PA:	SLVITOKKELK	IGRVCNTRCH	GVSTTAD
Orvza sativa	(1)		MATITT	OLRSALLS-PA	ASPSRRARRAP	SSVRCDSSAA	SSLSASA
Arabidoosis thaliana	(1)	MAN	VALOFSRLCV	RPDTFVRENHL:	SGSGSLRRRKA	LSVRCSSGDE	NAPSPSV
Rauvolfia verticillata	(1)	MA	ISLOFSGLST	RTADI ALP	-EPRIFRCWKP	VSVRCSAAGE	APAVSSS
Solanum tuberosum	(1)	MA	IPLOFSSIST	RTDLSLP	-ETRTERLPKP	FSVVRCSAGD	AVPSSSA
Consensus	(1)	MA	SLO LST	TD I.	A FR RKP	LSVRCSSG	S SS S
	(.)	61 70	00	10 1 00	100	110	120
Adamia vada astivas	(61)	<u>01</u> <u>70</u>		90	100		
Adonis_palaestina	(50)	AVESEFDAKSE.	RHNLTRSKNY	NRRGE'GHKDE'T	LELMNSEYTSD	VIKKLKENGN	EYSWGPV
Hevea_brasiliensis	(48)	AVESDE DAKVE	RHNLTRSKNY	NRRGFGHKEET	LELMNQEYTSD	IIKTLKENGN	QYKWGNV
vitis_vinitera	(51)	AVDSDE DAKVE	RHNLTRSKNY	NRKGEGHKDET	LELMNREYTSD	IIKTLKENGN	EYKWGNV
Artemsia_annua	(42)	SST-QFDAKVS	RHNLTRSENY	NRKGE'GHKKE'T	LELMSQEYFSD	IIKTLKENNY	EYTWGNV
Stevia_rebaudiana	(44)	ASGSDFDAKVF	RHNLTRSENY	NRKGFGHKKET	LELMNQEYTSD	IIKTLKENNN	EYTWGNV
Escherichia_coli	(1)						—————М
Ginkgo_biloba	(60)	SEPEQLDTKMF	RKNLTRSNNY	NRKGFGHKKET	LELMDQEYTSD	VVKTLKENNY	EYTWGNV
Oryza_sativa	(45)	SLDADFDKKQF	RHNLTRSDNY	NRKGFGHKKET	LELMSQEYTSD	VIKTLKENGN	QHTWGPV
Arabidopsis_thaliana	(52)	VMDSDFDAKVF	RKNLTRSDNY	NRKGFGHKEET	LKLMNREYTSD	ILETLKTNGY	TYSWGDV
Rauvoltia_verticillata	(48)	STESDFDAKKF	RHNLTRSKNY	NRRGFGLKEES	MELMNREYASD	IIQKLKDNGY	EYTWGNV
Solanum_tuberosum	(47)	TAESEFDAKVF	RKNLTRSANY	NRKGFGHKEAT	LELMNREYTSD	IIKKLKENGF	EYTWGNV
Consensus	(61)	A ESDFDAKVF	RHNLTRS NY	NRKGFGHKEET	LELMN EYTSD	IIKTLKENG	EYTWGNV
	(121)	121 130	0 14	0 150	160	170	180
Adonis palaestina	(110)	TVKLAESYGFC	WGVERAVOIA	YEARKOFPDEK	-IWITNEII	PTVNKRLEEM	EVKEIPV
Hevea_brasiliensis	(108)	TIKLAEAYGFC	WGVERAVOIA	YEARKOFPDEK	-IWITNEII <mark>H</mark> N	PT <mark>V</mark> NKRLEEM	NVQNIPV
Vitis vinifera	(111)	TVK <mark>LA</mark> EAYGFC	WGVERAVOIA	YEARKOFPEEK	-IWITNEII <mark>H</mark> N	PTVNORLAEM	EVKDIPI
Artemisia_annua	(101)	TVK <mark>LA</mark> EAFGFC	WGVERAVOIA	YEARKOFPDDK	-IWITNQII H N	PTVNKRLEEM	EVTDIPI
Stevia rebaudiana	(104)	TVK <mark>LA</mark> EAY <mark>GF</mark> C	WGVERAVQIA	YEARKQFPDEK	-IWITNEII	PT <mark>V</mark> NKRLEEM	EVKDIPV
Escherichia_coli	(2)	QILLANPRGFC	AGVDRAISIV	'EN <mark>A</mark> LAIYGAP-	-IYVRHEVV <mark>H</mark> N	RY <mark>V</mark> VDSLRER	GAIFI
Ginkgo_biloba	(120)	TVKLAEAYGFC	WGVERAVQIA	YE <mark>A</mark> RKQFPEER	-IWMTNEII <mark>HN</mark>	PTVNKRIEEM	KVQYIPV
Oryza_sativa	(105)	TVK <mark>LA</mark> EAY <mark>GF</mark> C	WGVERAVQIA	YEARKQFPDDR	-IWLTNEII <mark>hn</mark>	PTVNKRLEDM	GVQNIPV
Arabidopsis thaliana	(112)	TVK <mark>LA</mark> KAYGFC	WGVERAVQIA	YE <mark>A</mark> RKQFPEER	-LWITNEII <mark>hn</mark>	PT <mark>V</mark> NKRLEDM	DVKIIPV
Rauvolfia_verticillata	(108)	TVK <mark>LA</mark> EAY <mark>GF</mark> C	WGVERAVQIA	YE <mark>A</mark> RKQFPTER	-IWLTNEII <mark>hn</mark>	PTVNERLEEM	KVKEIPL
Solanum_tuberosum	(107)	TVK <mark>LA</mark> ESY <mark>GF</mark> C	WGVERAVQIA	YE <mark>A</mark> RKTVFQQR	GFWITNEII <mark>HN</mark>	PT <mark>V</mark> NRRLEDM	IDVKKNPL
Consensus	(121)	TVKLAEAYGFÇ	WGVERAVQIA	YEARKQFPDEK	IWITNEIIHN	PTVNKRLEEM	EVK IPV
	(101)	101 100	1 20	0 210	220	220	240
Adapia palaastina	(181)						
Adonis_palaestina	(169)	GDGKKHF DVVA.		GAAVSEMLILS			EKHKKGE
	(107)	GEGKKHF EVVD	SGDVVILPAF	GAAVEEMLILS		PWVSKVWNIV	EKHKKGE
	(170)	DDGQKQFEVVD.		GAAVDEMLILS	NKNVQIVDIIC	PWVSKVWNIV	EKHKKGE
Artemsia_arinua	(160)	DGGEKQFDVVD.		GAAVDEMRILS	DKEVQIVDIIC	PWVIKVWNVV	EKHKKGD
Slevia_rebaudiaria	(163)	KDGEKQFDVID.	KGDVVILPAF	GAAVNEMLILS		PWVSKVWISV	EKHKKGA
Eschenchia_coli	(58)	EQISEVP.	DGAILIFSAH	GVSQAVRNEAK	SRDLTVFDATC	PLVIKVHMEV	ARASRRG
Ginkgo_biloba	(179)	DEEGKREDVVD	KGDVVILPAF	GAAVHEMQYLS		PWVSKVWNTV	EKHKQGD
Uryza_sativa	(104)	DAGIKDF'DVVE	QGDVVVLPAF	GAAVEEMYTLN	EKKVQIVD'I IC	PWVSKVWNMV	EKHKKGD
Arabicopsis_thaliana	(1/1)	EDSKKQFDVVE		GAGVDEMYVLN		PWVIKVWNIV	EKHKKGE
	(107)	DDGEKQF'DVVD	QGDVVILPAF	GAGVDEMLTLS		PWVVKVWNSV	EKHKKGD
	(107)	LEGKKNFDVVD.		GAAVDEMLVLS		EWVIKVWNTV	ekhkkga
CUINSEINSUS	(101)	UUG KQE'DVVD.	ĸĠŊĸĸŦŗħ₩	GAAVDEMLTLS	UKINVQTVDTTC	FWVSKVWNTV	ькнккGD

(241)	241	250	260	270	280	290	300
Adonis_palaestina (229)	YT <mark>S</mark> IIH <mark>G</mark>	KYS H E E TIA	ASFAGKYII	VKNMDEAMYV	CDYILGGELN	GSSSDKQAL	lekf <mark>k</mark>
Hevea_brasiliensis (227)	YT <mark>S</mark> IIH <mark>G</mark>	KYS <mark>h</mark> eftia	T <mark>ASFAGK</mark> HII	VKNMEEAMYV	/CDYILGG <mark>Q</mark> LN	GSSSTKEAF	lekf <mark>k</mark>
Vitis_vinifera (230)	YT <mark>S</mark> IIH <mark>G</mark>	KYS <mark>H</mark> EETIA	T <mark>ASFAGKYII</mark>	VKNMAEAMYV	CDYILGG <mark>E</mark> LD	GSSSTREEF:	FEKF <mark>K</mark>
Artemisia_annua (220)	YT <mark>S</mark> VIH <mark>G</mark>	KHN <mark>H</mark> EETVA	TASFAGKFIV	VKNMDEATYV	CDYILGG <mark>K</mark> LN	GSSSTKEAF	MEKF <mark>k</mark>
Stevia_rebaudiana (223)	YT <mark>S</mark> IIH <mark>G</mark>	KYS <mark>H</mark> EETVA	TASFAGKYVI	VKNMDEATYV	/CDYILGG <mark>K</mark> LN	G <mark>SSSTKEAF</mark>	lekf <mark>k</mark>
Escherichia_coli (114)	EESILIG	hag <mark>h</mark> p e veg	MGQYS		NPE	GMYLVESP:	ddvw <mark>k</mark>
Ginkgo_biloba (239)	YT <mark>S</mark> IIH <mark>G</mark>	KYA <mark>H</mark> EETVA	TASFAGTYII	VKTIDEAAYV	CDYILDGKLN	GSSGTKAEF:	LQKF <mark>K</mark>
Oryza_sativa (224)	YT <mark>S</mark> IIH <mark>G</mark>	KYS <mark>h</mark> e f tva	T <mark>ASFAG</mark> TYII'	VKNIAEASYV	'CDYILGG <mark>Q</mark> LD	GSSSTKEEF:	lekf <mark>k</mark>
Arabidopsis_thaliana (231)	YT <mark>S</mark> VIH <mark>G</mark>	KYN <mark>H</mark> EETIA	I <mark>ASFAGKYII</mark>	VKNMKEANYV	CDYILGGQYD	GSSSTKEEFI	MEKF <mark>K</mark>
Rauvolfia_verticillata (227)	YT <mark>S</mark> IIH <mark>G</mark> I	KYS <mark>h</mark> eftia	I <mark>S</mark> SFAGKYII	VKNMKEAIYV	/CDYILGGQLD	GSSSTKEAF	MEKF <mark>K</mark>
Solanum_tuberosum(227)	YT <mark>S</mark> IIH <mark>G</mark>	KYA <mark>H</mark> EETVA	I ASFAGKYII'	VKNMAEATYV	CDYILGGKLD	G SSSTKEAFI	MQKF <mark>K</mark>
Consensus (241)	YTSIIHG	KYSHEETIA	TASFAGKYII	VKNM EA YV	CDYILGG LD	GSSSTKEAF	LEKFK
(301)	301	310	320	330	× 340	350	360
Adonis_palaestina (289)	YAISEGFI	OPDTDLI <mark>K</mark> T(GIAN <mark>QTT</mark> MLK(GE <mark>T</mark> EDIGKLL	EKTMMRKY <mark>G</mark> V.	ENINDHFIS	FNT <mark>IC</mark>
Hevea_brasiliensis (287)	YAVSKGFI	OPDVDLD <mark>K</mark> V(GIAN <mark>QTT</mark> MLK(GE <mark>T</mark> EEIGKLV	'EKTMMRKY <mark>C</mark> V	ENVNDHFISI	FNT <mark>IC</mark>
Vitis_vinifera (290)	FAISEGFI	OPDIDLS <mark>K</mark> V(GIAN <mark>QTT</mark> MLK(GE <mark>T</mark> EEIGKLV	'ERTMMRKY <mark>G</mark> V	ENVNNHFISI	FNT <mark>IC</mark>
Artemisia_annua (280)	YAVSEGFI	opdkdlv <mark>k</mark> a(GIAN <mark>QTT</mark> MLK(GE <mark>T</mark> EEIGKLI	ERTMMQKY <mark>C</mark> V.	EDVNNHFLSI	FNT <mark>IC</mark>
Stevia_rebaudiana (283)	YAVSNGFI	OPDTDLV <mark>K</mark> T(GVAN <mark>QTT</mark> MLK(GE <mark>T</mark> EEIGKLV	'ERTMMSKY <mark>G</mark> V	ENATEHFIS	FNT <mark>IC</mark>
Escherichia_coli (153)	LTVKN	EE <mark>K</mark> L:	SFMT <mark>QTT</mark> LSVI	DD <mark>T</mark> SDVIDAL	RKRFPKIV <mark>C</mark> P	Rl	KDD <mark>IC</mark>
Ginkgo_biloba (299)	NAVSKGFI	ophvalv <mark>k</mark> vo	GIAN <mark>QTT</mark> MLK(GE <mark>T</mark> EDIGKLV	'EKTMMHKF <mark>C</mark> V	ENINDHFIS	FNT <mark>IC</mark>
Oryza_sativa (284)	NAVSPGFI	OPDVDLV <mark>K</mark> V(GIAN <mark>QTT</mark> MLK(GE <mark>T</mark> EEIGKLV	'EKTMMRRF <mark>C</mark> V	ENVNDHFIA	FNT <mark>IC</mark>
Arabidopsis_thaliana (291)	YAISKGFI	opdndlv <mark>k</mark> v(GIAN <mark>QTT</mark> MLK(GE <mark>T</mark> EEIGRLL	ETTMMRKY <mark>C</mark> V	ENVSGHFISI	FNT <mark>IC</mark>
Rauvolfia_verticillata (287)	NAVSKGFI	OPDNDLL <mark>K</mark> V(GIAN <mark>QTT</mark> MLK(GE <mark>T</mark> EEIGKLI	ERTMMRKY <mark>C</mark> V	QNINDHFMSI	FNT <mark>IC</mark>
Solanum_tuberosum(287)	YAVSEGFI	opdvdlv <mark>k</mark> a(GIAN <mark>QTT</mark> MLK(GE <mark>T</mark> ADIGKLV	'ERTMMQKY <mark>C</mark> V	ENVNNHFVSI	FNT <mark>IC</mark>
Consensus (301)	YAVS GFI	OPD DLVKV	GIANQTTMLK	GETEEIGKLV	'EKTMMRKYGV	ENVNDHFIS	FNTIC
(361)	361	370	380	390	400	410	420
(361) Adonis_palaestina (349)	361 D <mark>AT</mark> QE <mark>R</mark> Q	_370 DAMFKLVEE	,380 KVDLI <mark>LV</mark> V <mark>G</mark> GI	,390 W <mark>NSSN</mark> TSH <mark>L</mark> ⊊	400 EISELRGIPS	410 SYW <mark>ID</mark> SETRI	420 GPGNK
(361) Adonis_palaestina (349) Hevea_brasiliensis (347)	361 DATQERQ DATQERQ	370 DAMFKLVEE DAMFKLVEE	_380 KVDLI <mark>LV</mark> VGGI KLDLI <mark>LV</mark> VGGI	,390 W <mark>NSSN</mark> TSHLÇ W <mark>NSSN</mark> TSHLÇ	400 EISELRGIPS EIAELRGIPS	410 SYWIDSETRI SYWIDSEQRI	420 GPGNK GPGNK
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350)	361 DATQERQ DATQERQ DATQERQ	,370 DAMFKLVEE DAMFKLVEE DAMYKLVEE	,380 KVDLI <mark>LVVG</mark> GI KLDLILVVGGI KLDVM <mark>LVVG</mark> GI	,390 W <mark>NSSN</mark> TSHLÇ W <mark>NSSN</mark> TSHLÇ	400 EISELRGIPS EIAELRGIPS EIAEDRGIPS	410 SYWIDSETRI SYWIDSEQRI SYW <mark>ID</mark> SEKRI	420 GPGNK GPGNK GPGNR
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340)	361 DATQERQ DATQERQ DATQERQ DATQERQ	370 DAMFKLVEE DAMFKLVEE DAMYKLVEE DAMYKLVDD	_380 KVDLILVVGG KLDLILVVGG KLDVMLVVGG KVDLMLV <mark>IG</mark> G	390 W <mark>NSSN</mark> TSHLQ WNSSNTSHLQ WNSSNTSHLQ F <mark>NSSN</mark> TSHLQ	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS	410 SYWIDSETRI SYWIDSEQRI SYWIDSEKRI SYWIDSEKRI	420 GPGNK GPGNK GPGNR GPGNR
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ	370 DAMFKLVEE DAMFKLVEE DAMYKLVEE DAMYKLVDD DAMYKLVDE	_380 KVDLILVVGGI KLDLILVVGGI KVDLMLVVGGI KVDLMLVIGGI KMDLMLVVGGI	,390 WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ FNSSNTSHLQ WNSSNTSHLQ	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS IPEERGIPS	,410 SYWID SETRI SYWID SEQRI SYWID SEKRI SYWID SEKRI SYWID SEKRI	420 GPGNK GPGNK GPGNR GPGNR GPGNR
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ YATTNR-	370 DAMFKLVEE DAMFKLVEE DAMYKLVED DAMYKLVDD DAMYKLVDE QEAVRALAE	_380 KVDLILVVGGI KLDLILVVGGI KVDLMLVVGGI KMDLMLVVGGI QAEVV <mark>LVVG</mark> SI	,390 WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ FNSSNTSHLQ WNSSNTSHLQ KNSSNSNRL2	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS IPEERGIPS LAQRMGKRA	410 SYWIDSETRI SYWIDSEQRI SYWIDSEKRI SYWIDSEKRI SYWIDTVERV FLIDDAKDI	420 GPGNK GPGNK GPGNR GPGNR GPGNR QEEWV
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ YATTNR- DATQERQ	370 DAMFKLVEE DAMFKLVEE DAMYKLVEE DAMYKLVDD DAMYKLVDE QEAVRALAE DAMHQLVKD	,380 KVDLILVVGG KLDLILVVGG KLDVMLVVGG KVDLMLVIGG KMDLMLVVGG QAEVVLVVGSI KLDLILVIGG	,390 WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ KNSSNSNRLA WNSSNTSHLQ	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS IPEERGIPS LAQRMGKRA	,410 SYWID SETRI SYWID SEQRI SYWID SEKRI SYWID SEKRI SYWID TVERV SFLID DAKDI SYWID SEERI	420 GPGNK GPGNR GPGNR GPGNR QEEWV GPGNM
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359) Oryza_sativa (344)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ YATTNR- DATQERQ DATQERQ	370 DAMFKLVEE DAMFKLVEE DAMYKLVDD DAMYKLVDE QEAVRALAE DAMHQLVKD DAMYQLVKE	380 KVDLILVVGG KLDLILVVGG KLDVMLVVGG KVDLMLVIGG KMDLMLVVGG QAEVVLVVGSI KLDLILVIGG KVDLILVVGG	,390 WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS IPEERGIPS IAELNGIPS IGELSGIPS	,410 SYWID SETRI SYWID SEQRI SYWID SEKRI SYWID TVERV SYWID SEERI SYWID SEERI SYWID SEERI	420 GPGNK GPGNR GPGNR GPGNR QEEWV GPGNM GPGNK
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359) Oryza_sativa (344) Arabidopsis_thaliana (351)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ YATTNR- DATQERQ DATQERQ DATQERQ	370 DAMFKLVEE DAMFKLVEE DAMYKLVDD DAMYKLVDE QEAVRALAE DAMHQLVKD DAMYQLVKE DAIYELVEE	380 KVDLILVVGGI KLDLILVVGGI KVDLMLVIGGI KMDLMLVVGGI QAEVVLVVGSI KLDLILVIGGI KVDLILVVGGI	,390 WNSSNTSHLQ WNSSNTSHLQ FNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ	400 ISELRGIPS IAELRGIPS IAEERKIPS IAEERKIPS IAELRGIPS IAELNGIPS IGELSGIPS	,410 SYWID SETRI SYWID SEQRI SYWID SEKRI SYWID TVERV SFLID DAKD I SYWID SEERI SYWID SEQRI SYWID SEQRI	420 GPGNK GPGNR GPGNR GPGNR QEEWV GPGNM GPGNK GPGNK
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359) Oryza_sativa (344) Arabidopsis_thaliana (351) Rauvolfia_verticillata (347)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ	370 DAMFKLVEE DAMFKLVEE DAMYKLVDD DAMYKLVDE QEAVRALAE DAMHQLVKD DAMYQLVKE DAIYELVEE DAMYKLVDQ	380 KVDLILVVGG KLDLILVVGG KVDLMLVIGG KMDLMLVVGG QAEVVLVVGSI KLDLILVIGG KVDLILVVGG SVDLMLVIGG	,390 WNSSNTSHLQ WNSSNTSHLQ FNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS IPEERGIPS IAELNGIPS IGELSGIPS ISEARGIPS	,410 SYWID SETRI SYWID SEQRI SYWID SEKRI SYWID TVERV FLID DAKDI SYWID SEERI SYWID SEQRI SYWID SEKRI SYWID SEKRI	420 GPGNK GPGNR GPGNR GPGNR QEEWV GPGNM GPGNK GPGNK GPGNR
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359) Oryza_sativa (344) Arabidopsis_thaliana (351) Rauvolfia_verticillata (347) Solanum_tuberosum (347)	361 DATQERQ DATQERQ DATQERQ DATQERQ YATTNR- DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ	370 DAMFKLVEE DAMFKLVEE DAMYKLVDD DAMYKLVDE QEAVRALAE DAMHQLVKD DAMYQLVKE DAIYELVEE DAMYKLVDQ DAMYKLVEQ	380 KVDLILVVGG KLDLILVVGG KVDLMLVVGG KMDLMLVVGG QAEVVLVVGSI KLDLILVIGG KVDLILVVGG SVDLMLVIGG KLDLMLVIGG	390 WNSSNTSHLQ WNSSNTSHLQ FNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS IPEERGIPS IAELNGIPS IGELSGIPS ISEARGIPS IAEERGIPS	,410 SYWID SETRI SYWID SEQRI SYWID SEKRI SYWID TVERV SFLID DAKDI SYWID SEERI SYWID SEKRI SYWID SEERI SYWID SEERI SYWID SEERI	420 GPGNK GPGNR GPGNR GPGNR QEEWV GPGNK GPGNK GPGNK GPGNR GPGNK
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359) Oryza_sativa (344) Arabidopsis_thaliana (351) Rauvolfia_verticillata (347) Solanum_tuberosum(347) Consensus (361)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ YATTNR- DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ	370 DAMFKLVEE DAMFKLVEE DAMYKLVDD DAMYKLVDE QEAVRALAE DAMHQLVKD DAMYQLVKE DAIYELVEE DAMYKLVDQ DAMYKLVEQ DAMYKLVEE	380 KVDLILVVGG KLDLILVVGG KVDLMLVVGG KVDLMLVVGG QAEVVLVVGS KLDLILVVGG KVDLILVVGG SVDLMLVIGG KLDLMLVVGG KLDLMLVVGG	390 WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS IPEERGIPS IAELNGIPS IGELSGIPS ISEARGIPS IAEERGIPS IAEERGIPS IAEERGIPS	,410 YWID SETRI YWID SEQRI YWID SEKRI YWID SEKRI YWID SEERI YWID SEERI YWID SEERI YWID SEERI YWID SEERI YWID SEERI YWID SEERI	420 GPGNK GPGNR GPGNR GPGNR QEEWV GPGNK GPGNK GPGNK GPGNK GPGNK
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359) Oryza_sativa (344) Arabidopsis_thaliana (351) Rauvolfia_verticillata (347) Solanum_tuberosum (347) Consensus (361) (421)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ 421	370 DAMFKLVEE DAMFKLVEE DAMYKLVDD DAMYKLVDE QEAVRALAE DAMHQLVKD DAMYQLVKE DAIYELVEE DAMYKLVDQ DAMYKLVEQ DAMYKLVEE 430	380 KVDLILVVGGU KLDLILVVGGU KVDLMLVVGGU KVDLMLVVGGU QAEVVLVVGSI KLDLILVIGGU KVDLILVVGGU SVDLMLVVGGU KLDLMLVVGGU KLDLMLVVGGU	390 WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ	400 ISELRGIPS IAELRGIPS IAECRGIPS IAECRGIPS IAECRGIPS IAELNGIPS IGELSGIPS IAECRGIPS IAECRGIPS IAECRGIPS IAECRGIPS IAECRGIPS	410 YWID SETRI YWID SEQRI YWID SEKRI YWID SEKRI YWID SEERI YWID SEERI	420 GPGNK GPGNR GPGNR GPGNR GPGNK GPGNK GPGNK GPGNK GPGNK GPGNK
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359) Oryza_sativa (344) Arabidopsis_thaliana (351) Rauvolfia_verticillata (347) Solanum_tuberosum (347) Consensus (361) (421) Adonis_palaestina (409)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ YATTNR- DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ 421 ISHKLNH	370 DAMFKLVEE DAMFKLVEE DAMYKLVDE DAMYKLVDE QEAVRALAE DAMHQLVKD DAMYQLVKE DAIYELVEE DAMYKLVEQ DAMYKLVEQ DAMYKLVEE 430 GELVETE-N	_380 KVDLILVVGGI KLDLILVVGGI KVDLMLVIGGI KMDLMLVVGGI KNDLILVVGGI KLDLILVIGGI SVDLMLVIGGI KLDLMLVVGGI KLDLMLVIGGI KLDLMLVVGGI MLPEGPVIIG	,390 WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS IAEERKIPS IAELNGIPS IAELNGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAE	410 YWID SETRI YWID SEQRI YWID SEKRI YWID SEKRI YWID SEERI YWID SEERI YWID SEERI YWID SEERI YWID SEERI YWID SEQRV YWID SE RI YWID SE RI YWID SE RI YWID SE RI YWID SEQRV YWID SE RI	420 GPGNK GPGNR GPGNR GPGNR GPGNK GPGNK GPGNK GPGNK GPGNK GPGNK 480 LA
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359) Oryza_sativa (344) Arabidopsis_thaliana (351) Rauvolfia_verticillata (347) Solanum_tuberosum(347) Consensus (361) (421) Adonis_palaestina (409) Hevea_brasiliensis (407)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ 421 ISHKLNH IAYKLNH	370 DAMFKLVEE DAMFKLVEE DAMYKLVDD DAMYKLVDE QEAVRALAE DAMHQLVKD DAMYQLVKE DAMYKLVEE DAMYKLVEQ DAMYKLVEE 430 GELVETE-N GELVEKE-N	_380 KVDLILVVGGI KLDLILVVGGI KVDLMLVIGGI KMDLMLVVGGI QAEVVLVVGSI KLDLILVIGGI KVDLILVVGGI SVDLMLVIGGI KLDLMLVIGGI KLDLMLVIGGI KLDLMLVIGGI FLPEGPVTIG	390 WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ FNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ VTSGASTPDK ITSGASTPDK	400 ISELRGIPS IAEDRGIPS IAEDRGIPS IAEERKIPS IAEERKIPS IAELNGIPS IGELSGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS	,410 YWID SETRI YWID SEQRI YWID SEKRI YWID TVERV FLID DAKDI YWID SECRI YWID SECRI	420 GPGNK GPGNR GPGNR GPGNR GPGNK GPGNK GPGNK GPGNK GPGNK GPGNK 480 LA VA
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359) Oryza_sativa (344) Arabidopsis_thaliana (351) Rauvolfia_verticillata (347) Solanum_tuberosum (347) Consensus (361) (421) Adonis_palaestina (409) Hevea_brasiliensis (407) Vitis_vinifera (410)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ YATTNR- DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ 421 ISHKLNH ISHKLNH	370 DAMFKLVEE DAMFKLVEE DAMYKLVDD DAMYKLVDE QEAVRALAE DAMYQLVKE DAMYQLVKE DAMYKLVEQ DAMYKLVEQ DAMYKLVEE 430 GELVETE-N GELVEKE-N	_380 KVDLILVVGG KLDLILVVGG KLDLMLVVGG KVDLMLVIGG QAEVVLVVGSI KLDLILVIGG KVDLILVVGG KLDLMLVVGG KLDLMLVVGG KLDLMLVIGG KLDLMLVIGG KLDLMLVIGG MLPEGPVTIG FLPEGPITIG	390 WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ FNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ VTSGASTPDK ITSGASTPDK VTSGASTPDK	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS IPEERGIPS IAELNGIPS IGELSGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS	410 YWID SETRI YWID SEQRI YWID SEKRI YWID SEKRI YWID TVERV FLID DAKDI YWID SECRI YWID SECRI Y	420 GPGNK GPGNR GPGNR GPGNR GPGNK GPGNK GPGNK GPGNK GPGNK GPGNK LA LA
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359) Oryza_sativa (344) Arabidopsis_thaliana (351) Rauvolfia_verticillata (347) Solanum_tuberosum (347) Solanum_tuberosum (347) Consensus (361) (421) Adonis_palaestina (409) Hevea_brasiliensis (407) Vitis_vinifera (410) Artemisia_annua (400)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ YATTNR- DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ 421 ISHKLNH ISHKLNH ISHKLMH IAYKLLH	370 DAMFKLVEE DAMFKLVEE DAMYKLVDD DAMYKLVDE QEAVRALAE DAMHQLVKD DAMYQLVKE DAMYKLVEE DAMYKLVEE 430 GELVETE - N GELVEKE - N GELVEKE - N GELVEKE - N	_380 KVDLILVVGGI KLDLILVVGGI KVDLMLVVGGI KVDLMLVVGGI QAEVVLVVGSI KLDLILVIGGI KVDLILVVGGI KLDLMLVVGGI KLDLMLVVGGI KLDLMLVVGGI FLPEGPITIG WLPEGPITIG	390 WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ FNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ VTSGASTPDK VTSGASTPDK VTSGASTPDK VTSGASTPDK	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS IPEERGIPS IAELNGIPS IGELSGIPS IAEERGIPS	410 YWID SETRI YWID SEQRI YWID SECRI YWID SEKRI YWID SEKRI YWID SECRI YWID SECRI Y	420 GPGNK GPGNR GPGNR GPGNR GPGNK GPGNK GPGNK GPGNK GPGNK 480 LA LA LA LV
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359) Oryza_sativa (344) Arabidopsis_thaliana (351) Rauvolfia_verticillata (347) Solanum_tuberosum (347) Consensus (361) (421) Adonis_palaestina (409) Hevea_brasiliensis (407) Vitis_vinifera (410) Artemisia_annua (400) Stevia_rebaudiana (403)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ 421 ISHKLNH IAYKLNH ISHKLMH IAYKLLH IAYKTMH	370 DAMFKLVEE DAMFKLVEE DAMYKLVDD DAMYKLVDE QEAVRALAE DAMHQLVKD DAMYQLVKE DAMYQLVKE DAMYKLVDQ DAMYKLVEE 430 GELVETE-N GELVEKE-N GELVEKE-N GELVEKE-N	_380 KVDLILVVGGU KLDLILVVGGU KVDLMLVVGGU KVDLMLVVGGU QAEVVLVVGSI KLDLILVIGGU KVDLILVVGGU KLDLMLVVGGU KLDLMLVVGGU KLDLMLVVGGU FLPEGPITIGU WLPEGPITIGU WLPKGPLTIGU	390 WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ FNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ VTSGASTPDK ITSGASTPDK VTSGASTPDK ITSGASTPDK ITSGASTPDK	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS IAEERGIPS IAELNGIPS IGELSGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS IAEERGIPS A60 AVEDALVKVF VVEDVLIKVF AVEDVLIKVF	,410 YWID SETRI YWID SEQRI YWID SEKRI YWID SEKRI YWID SEKRI YWID SEERI YWID SEERI	420 GPGNK GPGNR GPGNR GPGNR QEEWV GPGNK GPGNK GPGNK GPGNK GPGNK 480 LA LA LA FA
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359) Oryza_sativa (344) Arabidopsis_thaliana (351) Rauvolfia_verticillata (347) Solanum_tuberosum (347) Consensus (361) (421) Adonis_palaestina (409) Hevea_brasiliensis (407) Vitis_vinifera (410) Artemisia_annua (400) Stevia_rebaudiana (403) Escherichia_coli (257)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ 421 ISHKLNH IAYKLNH IAYKLNH IAYKLH IAYKLH IAYKTMH	370 DAMFKLVEE DAMFKLVEE DAMYKLVDD DAMYKLVDE QEAVRALAE DAMHQLVKD DAMYQLVKE DAMYQLVKE DAMYKLVDQ DAMYKLVEQ DAMYKLVEE 430 GELVETE-N GELVEKE-N GELVEKE-N GELVEKE-N GELVEKE-N VTAGASAPD	_380 KVDLILVVGG KLDLILVVGG KVDLMLVVGG KVDLMLVIGG QAEVVLVVGSI KLDLILVIGG KVDLILVGG KVDLILVGG KLDLMLVVGG KLDLMLVVGG KLDLMLVIG KLDLMLVIG KLDLMLVG KLDLMLVIG KLDLMLV KLDLMLVIG KLDLMLV KLDLMLV K	390 WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ FNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ VTSGASTPDK UTSGASTPDK VTSGASTPDK UTSGASTPDK QQLGGGEAIE	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS IAEERGIPS IAELNGIPS IAEERGIPS	,410 YWID SETRI YWID SEQRI YWID SEKRI YWID SEKRI YWID SEKRI YWID SEERI YWID SEERI YWID SEERI YWID SEQRV YWID SEQRV YWID SEQRV YWID SEQRV DIKREQLLQ DIKREQLQ DIKREALQ EIKREEALQ EIKREESLQ EVFKELRVD	420 GPGNK GPGNR GPGNR GPGNR QEEWV GPGNK GPGNK GPGNK GPGNK GPGNK 480 LA
(361) Adonis_palaestina (349) Hevea_brasiliensis (347) Vitis_vinifera (350) Artemisia_annua (340) Stevia_rebaudiana (343) Escherichia_coli (198) Ginkgo_biloba (359) Oryza_sativa (344) Arabidopsis_thaliana (351) Rauvolfia_verticillata (347) Solanum_tuberosum (347) Consensus (361) (421) Adonis_palaestina (409) Hevea_brasiliensis (407) Vitis_vinifera (410) Artemisia_annua (400) Stevia_rebaudiana (403) Escherichia_coli (257) Ginkgo_biloba (419)	361 DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ DATQERQ 421 ISHKLNH ISHKLNH ISHKLNH ISHKLNH IAYKLLH IAYKLNH	370 DAMFKLVEE DAMFKLVEE DAMYKLVDD DAMYKLVDE QEAVRALAE DAMHQLVKD DAMYQLVKE DAIYELVEE DAMYKLVDQ DAMYKLVEQ DAMYKLVEE 430 GELVETE-N GELVEKE-N GELVEKE-N GELVEKE-N VTAGASAPD GELVEKE-N	380 KVDLILVVGGV KLDLILVVGGV KVDLMLVGGV KVDLMLVGGV KVDLMLVGGV KLDLILVGGV KLDLILVGGV KLDLMLVVGGV KLDLMLVVGGV KLDLMLVGGV KLDLMLVGGV KLDLMLVGGV MLPEGPITIGV MLPEGPITIGV MLPEGPITIGV MLPEGPITIGV MLPEGPITIGV MLPEGPITIGV MLPEGPITIGV MLPEGPITIGV MLPEGPITIGV MLPEGPITIGV MLPEGPITIGV MLPEGPITIGV	390 WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WNSSNTSHLQ WSSNTSHLQ VTSGASTPDK UTSGASTPDK UTSGASTPDK UTSGASTPDK UTSGASTPDK UTSGASTPDK UTSGASTPDK	400 ISELRGIPS IAELRGIPS IAEDRGIPS IAEERKIPS IPEERGIPS IAELNGIPS IAELNGIPS ISEARGIPS IAEERGIPS	,410 YWID SETRI YWID SEQRI YWID SEKRI YWID SEKRI YWID SEERI YWID SEERI YWID SEERI YWID SEERI YWID SEQRV YWID SEQRV YWID SEQRV YWID SEQRV DIKREQLLQ DIKREEALQ EIKREEALQ EIKREESLQ EVFKELRVD QIKQEETLP	420 GPGNK GPGNR GPGNR GPGNR QEEWV GPGNK GPGNK GPGNK GPGNK 480 LA
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Figure 2. Multi-alignment of amino acid sequences of RvHDR and other HDRs. 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. *Stars* mark the position of conserved cysteine residues.



Figure 3. A phylogenetic tree of HDRs from different organisms including plants, algaes and bacteria constructed by the neighbor-joining method on MEGA 3.0. HDRs from plants were marked with ., HDRs from algaes marked with ., and the others from bacteria marked witho. The numbers on the branches represented bootstrap support for 1000 replicates. The sequences used were listed bellow with Accession number: Picrorhiza kurrooa, ABM89226.1; Vitis vinifera, CAO47671.1; Hevea brasiliensis, BAF98297.1; Adonis palaestina, AAG21984.1; Solanum tuberosum, ABB55395.1; Arabidopsis thaliana, AAN87171.1; Artemisia annua, ABY57296.1; Stevia rebaudiana, ABB88836.2; Oryza sativa, NP 001051167.1; Ginkgo biloba, ABC84344.1; Acaryochloris marina, YP 001519239.1; Thermosynechococcus elongatus, NP_681832.1; Microcystis aeruginosa, CAO90213.1; Cyanothece, ZP_01731309.1; Anabaena variabilis, YP_323455.1; Nostoc, NP_485028.1; Bacillus licheniformis, YP_079844.1; Clavibacter michiganensis, YP 001222973.1; Tropheryma whipplei, CAD67323.1; Bifidobacterium longum, NP_696525.1; Rhodobacter sphaeroides, ABN75415.1; Psychromonas ingrahamii, ABM04955.1; Pseudoalteromonas atlantica, ABG41681.1; Escherichia coli, AAL38655.1.

participate in the coordination of the iron-sulfur bridge proposed to be involved in catalysis (Seemann et al., 2002; Wang et al., 2008). The position of one of these cysteine residues was not conserved in the *E. coli* protein (Figure 2), but it was possible that the cysteine at position 263 in the *E. coli* sequence might participate in the [4Fe-4S] coordination. Using MEGA 3.0 based on CLUSTAL X alignments, a phylogenetic tree of HDRs was constructed from different organisms including plants, algaes and bacteria. The result demonstrated that HDRs were derived from an ancestor gene and evoluted into three groups including plants, algaes and bacteria HDR group. *RvHDR* had higher identity with plant HDRs than bacterium and algae HDRs (Figure 3). All the analysis results strongly suggest that *RvHDR* is a plant HDR protein involved in the mevalonate-independent biosynthesis.

Expression profile analyses

To investigate the expression profile of *RvHDR* in different tissues including roots, stems, leaves, flowers and fruits of *R. verticillata*, total RNAs were isolated from different tissues and subjected to semi-quantitative one-step RT-PCR using fex*RvHDR* and rex*RvHDR* as



Figure 4. Expression profile of *RvHDR* in different tissues of *R. verticillata.* Total RNA samples were isolated from roots, stems, leaves, flowers and fruits respectively, and subjected to Semi-quantitative one-step RT-PCR analysis (upper panel). *18S rRNA* gene was used as the control to show the normalization of the amount of templates in PCR reactions (lower panel).



Figure 5. Expression profile of *RvHDR* under induction by elicitors including UV, 100 µM MeJA, 50 µM ABA and 100 mg.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.

primers. The house-keeping gene (18S rRNA gene) expression in all the detected tissues was used as internal control that showed no significant difference. The result showed *RvHDR* expression could be detected in all tissues, suggesting that *RvHDR* is constitutively expressed but at different levels in different organs. Furthermore, the highest expression level of *RvHDR* was found in flowers of *R. verticillata*, followed by in fruits (Figure 4).

To investigate the induction by various elicitors, onestep RT-PCR analyses were carried out to monitor the changes of *RvHDR* expression levels upon various elicitor treatments including 100 μ M MeJA, 100 mg.L⁻¹ ASA, 50 μ M ABA and UV. The result showed that the expression levels of *RvHDR* were all strongly increased by MeJA, ASA, ABA and UV treatments, among which the highest transcript level of *RvHDR* was found by UV treatment (Figure 5). The result was consistent with the HDR gene expression in Camptotheca acuminata (Wang et al., 2008).and Arabidopsis thaliana (Hsieh et al., 2005) and suggested that *RvHDR* was a highly-regulated gene for basic physiological and biochemical processes in *R. verticillata*.

RvHDR complements the E. coli HDR mutant

To test whether the HDR protein of R. verticillata has similar enzymatic activity to its E. coli counterpart, we used a complementation assay with an E. coli HDR mutant. In E. coli HDR mutant strain MG1655 ara<>HDR the endogenous HDR gene was replaced by a kanamycin-resistant cassette and a single copy of HDR was present on the chromosome under the control of the PBAD promoter (McAteer et al., 2001). The E. coli HDR mutant cannot form colonies on LB medium in the absence of arabinose (Figure 6), because the HDR gene is essential for survival. When pQE30-RvHDR plasmid containing the coding region of RvHDR was transformed into the E. coli HDR mutant strain MG1655 ara<>HDR, this bacteria can restore successfully on the medium containing 0.2% Glc but cannot by transforming the empty pQE30 vector.

The genetic complementation strategy was applied to identify the function of *RvHDR* in mutant *E-coil* strain MG1655 ara<>HDR. The HDR gene is an essential gene for survival of *E. coil*. In the present study, *RvHDR* was introduced into mutant *E-coil* strain MG1655 ara<>HDR



Figure 6. *R. verticillata* HDR complements the *E. coli* HDR mutant. The *E. coli* HDR mutant strain MG1655ara<>HDR was able to grow on LB media containing 0.2% Ara, but not on media containing 0.2% Glc (right). After transformation with the *RvHDR* coding region (pQE30-*RvHDR*) and, as a control, with the empty vector (pQE30) alone, the resulting strains were tested for growth on media containing 0.2% Glc and 0.5 mM IPTG (left). Expression of RvHDR protein successfully restored the growth of the *E. coli* HDR mutant (below left).

and over expressed, and then the mutant was rescued by *RvHDR* in media without arabinose. It was confirmed that the HDR protein of *R. verticillata* had similar enzymatic activity to its *E. coli* counterpart.

DISCUSSION

HDR catalyzes the last reaction of the methylerythritol phosphate (MEP) pathway, a branching step that separately produces isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) in a ratio of 5:1 (Altincicek et al., 2002) and is the ideal target for metabolic engineering of the isoprenoid biosynthetic pathway. In our study, we have successfully isolated and characterized the RvHDR cDNA from R. verticillata for the first time. The RvHDR cDNA contained a 1389-bp ORF coding for protein RvHDR with 462-amino acid residues. The further bioinformatics analysis indicated that RvHDR contained a 37-amino transit peptide that directed it to plasmid, the result was consistent with the fact that the precursor of TIAs was synthesized in plastids (Yamazaki et al., 2003). The existence of expression of RvHDR in all detected tissues but in different levels showed RvHDR could be an essential gene that was also highly regulated like the HDR genes from Arabidopsis (Hsieh et al., 2005), Ginkgo and Pinus (Kim et al., 2008). In recent years, many studies showed some elicitors were the excellent stimulators of alkaloid biosynthesis exogenously applied to cell suspension cultures, such as MeJA (Sheludo et al., 1998), ASA (Gong et al., 2005).

Our present study indicated that all the elicitors including MeJA, ASA, ABA and UV could up-regulate the expression of RvHDR. The results provide direct evidence that *RvHDR* is an elicitor-responsive gene and can be effectively elicited at least at the transcription level. Finally, the RvHDR was been introduced into E. coil HDR mutant strain MG1655 ara<>HDR and over expressed, and then the mutant was rescued by RvHDR in the media without ara. The result of genetic complementation demonstrated that RvHDR gene reported here did encode the active enzyme HDR. Cloning, characterization, and functional identification of *RvHDR* will facilitate the understanding of the biosynthesis of TIAs including reserpine and ajmalicine and also promote metabolic engineering of the TIAs biosynthetic pathway in *R. verticillata*.

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