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3-Hydroxy-3-methylglutaryl-coenzyme A reductase of Vietnamese ginseng: Structure, promoter identification, and expression analysis

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ABSTRACT

Vietnamese ginseng (*Panax vietnamensis* Ha et Grushv.), a traditional Vietnamese medicinal herb, has been demonstrated to produce triterpenoids with anti-cancer effects. However, the mechanism of triterpenoid biosynthesis in this species remains poorly understood. 3-Hydroxy-3-methylgutary coenzyme A reductase (HMGR, EC 1.1.1.34) catalyzes the NAD(P)H-dependent reduction of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonate, the first committed step in the isoprenoid pathway, which generates the majority of modern natural products. To investigate the triterpenoid's biosynthetic mechanism, the HMG-CoA reductase (named *PvH_HMGR*) gene of Vietnamese ginseng was identified. A BLASTp search against the non-redundant (nr) protein sequence showed that PvH_HMGR has 88.13–98.98% identity with HMGRs from different plant species, and multi-alignment comparison analysis showed the presence of two motifs, each corresponding to HMG-CoA-binding and NADP(H)-binding. PvH_HMGR belongs to Class I HMG-CoA reductase, according to the conserved domain analysis. The secondary structure of the enzyme, as well as its hydrophilicity scales, transmembrane topology, and core promoter, were predicted and described. Three-dimensional enzyme modeling was predicted and annotated by various software with high coverage and confidence. Furthermore, the abiotic stress induced by the various concentrations of salts significantly improved the expression of PvH_HMGR in the adventitious root of Vietnamese ginseng *in vitro*.

1. INTRODUCTION

Vietnamese ginseng, commonly referred to as Ngoc Linh ginseng and scientifically known as *Panax vietnamensis* Ha et Grushv., is a valuable ginseng species native to Vietnam. Vietnamese ginseng, a herbaceous perennial that is a member of the Araliaceae family, was first discovered at Ngoc Linh mountain in Gia Lai-Kontum, Vietnam, in 1973 [1,2]. Its native distribution is limited to the Ngoc Linh and Hoang Lien Son mountain ranges, and they are cultivated in two conservation areas in the provinces of Quang Nam and Kon Tum on the Ngoc Linh mountain's slopes [3,4]. Vietnamese ginseng was utilized by ethnic minorities as traditional medicine for the enhancement of physical strength and treatment of a variety of illnesses before it was discovered [1,2,5].

Vietnamese ginseng has been shown to have obvious pharmacological effects in the treatment of anti-stress, anti-oxidation, anti-inflammatory, anti-aging, and anti-cancer [6,7]. Saponins are the main group of biologically active compounds in Vietnamese ginseng, with more than 52 different derivatives [7-9]. Due to their significant medicinal and commercial worth, as well as overexploitation, they are included in the Vietnam Red Book as a rare plant species that must be preserved [10].

Saponins are glycosides of triterpenoids or steroids, of which the triterpenoid saponins are dominant in Vietnamese ginseng [11,12]. Triterpenoid saponins offer numerous other advantages outside of defending plants from pathogenic bacteria and herbivores, such as enhancing health through immunomodulation, hypocholesterolemia, anti-coagulation, anti-cancer, hepatoprotection, anti-inflammatory properties, antioxidants, and many more [13,14].

Terpenoids are the largest group of plant secondary metabolites and are biosynthesized by two pathways in plants: The mevalonate (MVA) pathway in the cytosol and the non- MVA pathway (MEP) in the plastids [15]. The biosynthesis of terpenoids by the MEP begins with two acetyl-CoAs that are condensed to form acetoacetyl-CoA under the catalysis of acetoacetyl-CoA thiolase (AACT, EC 2.3.1.9). Acetoacetyl-CoA is then catalyzed to form 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) by HMGCoA synthase (HMGS, EC 2.3.3.10). HMG-CoA is catalyzed to form mevalonic acid by HMG-CoA reductase (HMGR, EC 1.1.1.34), followed by enzymatically catalyzed reactions to form other precursors, and finally triterpenoid saponins. Recent research on the MEP has primarily focused on discovering novel pathways [16], identifying and characterizing important enzymes, and improving their expression in plant cells and tissue culture [17-19]. Genes from the

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Panax genus that encode key enzymes in the MEP have also been studied [Table 1]. HMGR is the rate-limiting enzyme, which is one of the key elements involved in the catalytic reactions of terpenoid biosynthesis in the MEP in plants [20]. However, the literature review showed that the HMGR genes from *P. vietnamensis* have not yet been cloned and their structures have not been analyzed. The use of sodium chloride or sodium carbonate as abiotic elicitors in Vietnamese ginseng cultures *in vitro* has not yet been reported. Although some studies have indicated that the elicitors can improve the expression levels of genes involved in the production of triterpenoid saponin in plant cells and tissues [21-25].

In the previous studies, we identified three isoforms of squalene epoxidase (SE) in the MEP of Vietnamese ginseng [19]. In the present work, we first reported on molecular cloning, characteristic analysis, 3D structural modeling, and annotation of the HMGR from Vietnamese ginseng. The study also wanted to improve the expression level of the key gene in the adventitious root culture of this medicinal species using salt elicitation, which had not been done in any previous studies.

2. MATERIALS AND METHODS

2.1. Plant Material

Vietnamese ginseng that is 6 years old was provided by Mr Tran Ut (Director of the Center for Developing Ngoc Linh Ginseng and Medicinal Herbs, Quang Nam Province, Vietnam) and identified by the Institute of Ecology and Biological Resources of Vietnam. The plants were collected according to the relevant guidelines and regulations of Vietnam. The *in vitro* adventitious roots of Vietnamese ginseng were prepared by Prof. Duong Tan Nhut (Tay Nguyen Institute of Scientific Research, Vietnam Academy of Science and Technology) and subcultured on Schenk and Hildebrandt (SH) medium [26], supplemented with 30 g/L sucrose, 4 mg/L indolebutyric acids, and 0.5 mg/L kinetin. *In vitro* cultures were incubated at 25 ± 2°C with a shaking speed of 100 rpm in the dark [25].

2.2. Molecular Cloning

The specific primers for PCR amplification of the *PvH_HMGR* gene were designed by the Primer 3 web tool based on the predicted nucleotide sequences of this gene [19]. The primers were then specifically checked by the Primer-BLAST tool against the non-redundant (NR) database limited to Vietnam ginseng. The following primers (5'-3') of forward ATGGAC GTTCGCCGGCGAC and reverse GTAATTAGGAAGAGAGCTTGGAGACATC were used for cloning. Total RNA was extracted from the root tissues by the Gene JET Plant RNA Purification Kit (Thermo Scientific, USA). The putative *PvH_HMGR* gene was amplified by the RT-PCR method. The PCR reactions were performed under the following conditions: 94°C for 5 min; followed by 30 cycles of 94°C for 30 s, 58°C for 45 s, and 72°C for 2 min; and a final extension at 72°C for 10 min. The PCR products were subcloned into a pGEM T-easy vector (Promega, USA) and then sequenced by the Sanger method (FirstBase, Malaysia).

2.3. Bioinformatic Analysis

The full-length sequence of the putative *PvH_HMGR* gene was subjected to a BLAST search against the NR databases to find out the close homologs. In addition, the *HMGR* gene was predicted using databases from the *P. vietnamensis* (SRA accession number: PRJNA665343) [19] and *P. vietnamensis* var. fuscidiscus (SRA accession number: SRR1198998) [12]. Based on nrBLAST results, the best templates and predicted *PvH HMGR* sequences were multi-aligned by the CLC Sequence Viewer (Qiagen) to identify the presence of conserved motifs. The introns and exons were predicted by comparing the cloned cDNA and predicted DNA sequences of the *PvH_HMGR* gene. The transmembrane helices of enzymes were predicted using the DeepTMHMM server. The hydrophobicity of PvH_HMGR enzymes was analyzed by ProtScale using the amino acid scale of Kyte-Doolittle. The secondary structures of enzyme sequences were predicted using the LOMET and I-TASSER tools. The

Table 1: Identification of some key enzymes in the MVA pathway of *Panax* genus.

S. No	Gene	Enzymes and EC number	*Panax* species (GenBank)
1	AACT	Acetyl-CoA C-acetyltransferase EC: EC 2.3.1.9	*P. vietnamensis* Ha et Grushv. (MZ272018.1) and *P. notoginseng* (KJ804173.1).
2	HMGS	3-hydroxy-3-methylglutaryl coenzyme-A synthase EC: 2.3.3.10	*P. notoginseng* (KJ804167.1; KP702301.1), *P. ginseng* (GU565098.1)
3	HMGR	3-hydroxy-3-methylglutaryl coenzyme-A reductase EC: 1.1.1.34	*P. notoginseng* (KP702300.1, KJ804166.1, KJ578757.1, KP702301.1), *P. ginseng* (KM386694.1, KM386695.1, GU565097.1, KJ939263.1), *P. quinquefolius* (FJ755158.2)
4	FPS	Farnesyl diphosphate synthase EC: 2.5.1.10	*P. ginseng* (DQ087959.1), *P. vietnamensis* (MZ272019.1), *P. notoginseng* (MK757455.1, KF597527.1, KJ804175.1, KC953034.1, KC524468.1), *P. quinquefolius* (GQ401664.1), *P. japonicas* (KP684141.1), *P. sokpayensis* (JZ822887.1)
5	SS	Squalene synthase EC: 2.5.1.21	*P. quinquefolius* (KT869136.1, GU997681.1, AM182457.1, AM182456.1, KC524471.1, KC524469.1), *P. notoginseng* (KT123897.1, DQ186630.1, KC953032.1, KC422650.1, KT123898.1), *P. sokpayensis* (KT936528.1), *P. ginseng* (EU502717.1, GU183406.1, GQ468527.2, AB010148.1, KP689323.1, KP689322.1, KP689321.1, KP689320.1, KP689319.1, KP689318.1, KP689317.1, KP689316.1, KP689315.1, KP689314.1, KJ939264.1), *P. japonicas* (KP890782.1)
6	SE	Squalene epoxidase EC: 1.14.14.17	*P. notoginseng* (DQ457054.1, KT123898.1, JX625132.1, DQ386734.1, KC953033.1, KC422651.1, KT123897.1), *P. vietnamensis* Ha et Grushv. (MW258700.1, MW258699.1, MW258698.1), *P. sokpayensis* (KT936529.1), *P. vietnamensis* var. fuscidiscus (KJ946469.1, KJ946468.1, KJ946467.1) *P. ginseng* (FJ393274.2, AB122078.1, AB003516.1), *P. japonicas* (MK603118.1).
7	PPDS	Protopanaxadiol synthase EC: 14.14.121	*P. sokpayensis* (MF682462.1), *P. notoginseng* (KJ995703.1), *P. ginseng* (JX036031.1), *P. vietnamensis* Ha et Grushv. (ON693838)
8	PPTS	Protopanaxatriol synthase EC: 1.14.14.121	*P. sokpayensis* (MF682463.1), *P. ginseng* (JX036031.1), *P. quinquefolius* (KC190491.1), *P. notoginseng* (KF935232.1), *P. vietnamensis* Ha et Grushv. (ON693839)

P. vietnamensis: Panax vietnamensis, P. notoginseng: Panax notoginseng, P. sokpayensis: Panax sokpayensis, P. quinquefolius: Panax quinquefolius, P. ginseng: Panax ginseng

structural and functional domains in the deduced PvH_HMGR enzyme molecule were identified by the SMART and HMMSCAN tools. The enzyme structure model was analyzed and compared by aligning the predicted models from the various servers (PHYRE2, SWISS-MODEL, I-TASSER, and AlphaFold 2.0) and simulated with Pymol v2.5.2. The phylogenetic tree was generated by MEGA X following the neighbour-joining method (NJ) with 1000 bootstrap replicates and visualized by iTOL. The signal peptide of the enzyme was predicted by SignalP 6.0 based on Neural Networks and Hidden Markov models databases of eukaryotes with the best confidence value. The theoretical isoelectric point (pI) was estimated by the Compute pI/MW application from Expasy. Furthermore, the 500 bp of promoter regions upstream of the translation start site of the *PvH_HMGR* gene were extracted from the draft assembly genome's predicted PvH_scaffold0913 (756124-759846 nt), which was predicted and annotated by various bioinformatics tools, including PlantPAN 3.0, TSSPlant, Plantprom, PlantCARE, and CpG islands.

2.4. Elicitation and Semi-quantitative RT-PCR

The adventitious root of Vietnamese ginseng was treated with NaCl (0.05, 0.1, 0.15, and 0.2%) and Na_2CO_3 (0.25, 0.5, 0.75, and 1%) [24] at the beginning or after 20 days of culture. After 56 days of culture [25], including the time for elicitation, the root biomass was collected, and it was then immediately frozen with liquid nitrogen in preparation for total RNA extraction using the GeneJET Plant RNA Purification Kit (ThermoFisher, USA). The first-strand cDNA was synthesized by the RevertAid First-Strand cDNA Synthesis Kit (Thermo Scientific, USA) according to the manufacturer's instructions. The oligonucleotide primers (5'-3') GGTCTCCAAAGGCGTCCA (forward) and CCAAGGCTGCCACATTA-GTC (reverse) were used for gene expression analysis. Two microliters of the first-strand cDNA were then used for PCR amplification with the following conditions: 94°C for 5 min; followed by 35 cycles of 94°C for 30 s, 58°C for 45 s, and 72°C for 2 min; and a final extension at 72°C for 10 min. The *GAPDH* gene was utilized as the internal control gene for measuring and reducing the errors between the samples in RT-PCR expression analyses [12].

3. RESULTS

3.1. Molecular Cloning of PvH_HMGR

The putative *PvH_HMGR* gene's cDNA sequence (1.773 bp long), which was obtained by RT-PCR amplification, was compared to related genes from other *Panax* species. The results showed that this sequence is 100% similar to the coding DNA sequence of the predicted gene from the assembled genome of Vietnamese ginseng. In addition, it was inferred from the comparison that the full-length *PvH_HMGR* gene may also include four exons and three introns. The deduced amino acid sequence of the *PvH_HMGR* gene consists of a polypeptide chain of 590 amino acids with an estimated molecular weight (MW) of about 63.11 kDa.

3.2. Characterization of PvH_HMGR Enzyme

The structural and functional domain analysis of the protein encoded by the *PvH_HMGR* gene indicated that it is the enzyme HMG-CoA reductase with a conserved domain (cd00643) from amino acids (aa) 203–580, and the active site was presented at 269 and 575 aa. Furthermore, the PvH_HMGR also contains two HMG-CoA binding motifs (EMPVGYVQIP and TTEGCLVA) as well as two NADP(H)-binding motifs (GTVGGG and DAMGMNM). The result of transmembrane helix analysis indicated PvH_HMGR has two transmembrane domains (43–57 and 78–96 aa) and no signal peptide [Figures 1 and 2]. The subcellular localization analysis of the PvH_HMGR revealed that its membrane domain contributes to the endoplasmic reticulum (ER) morphogenesis in Vietnamese ginseng cells. Also shown by the analysis was the presence of typical hydrophobic regions in the *PvH_HMGR*-deduced amino acid sequences. With a high localization probability of 0.6923, these regions, which contain the RRR ER-retention motif, play a crucial role in anchoring enzymes to the ER. The PvH_HMGR enzyme was also shown to contain a large number of hydrophilic domains, including ones from aa 34 to 56, 68-104, 180-187, 218-223, 233-255, 283-296, 326-332, 344-351, 417-424, 433-460, 463-473, 486-492, 522-532, and 546-569. The secondary structure of PvH_HMGR contains 42.88% of alpha-helices with 38.14% of random coils, 14.41% of extended strands, and 4.58% of beta-turns. In addition, the average theoretical isoelectric point (pI) of the enzymes was also estimated at 6.66. The deduced amino acid of PvH_HMGR was searched in the STRING database 2021 to investigate the protein-protein interaction network. The analysis showed that PvH_HMGR is similar to 3-hydroxy-3-methylglutaryl coenzyme A reductase isoform 1, with various percentages (72.4–88%) identified in some plants.

3.3. Homology-modeling of the PvH_HMGR Structure

PvH_HMGR's 3D protein structure was predicted by several servers and software, including PHYRE2, I-TASSER, and AlphaFold-2. Specifically, PHYER2 modeled 394 residues of PvH_HMGR (67%), with 100.0% confidence, using the single highest scoring template of human HMG-CoA reductase (PDB No.: 1HWJ). Besides, I-TASSER predicted the protein structure (70% coverage) using the best template of human HMG-CoA reductase (PDB No.: 1DQ8) with the highest Z-score significance (4.61). AlphaFold-2 assigns a confidence score to each residue based on a pLDDT value ranging from 0 to 100. The results of Alphafold-2 structure prediction revealed that 70% of residues have very high confidence (pLDDT value >90) and approximately 3.9% have low confidence (residue positions 50–70), which are found at the N and T-termini. In addition, the predicted binding site of PvH_HMGR to some ligands such as HR2, COA, ADP, and NAP is depicted in Figure 2. Furthermore, the active site of the enzyme was predicted at residues 269 and 575. The predicted homology model with high similarity to human HMG-CoA reductase would provide important information for further studies on the biological function and kinetics of this enzyme.

3.4. The Core Promoter of PvH_HMGR Prediction

Several putative *cis*-elements were identified in the partial promoter of PvH_HMGR [Figure 3]. One MYC element was identified in the promoter. In addition, motifs involved in hormonal regulation, including ABRE, ABRE3a, and the TCA element, were identified in the promoter regions of the gene. These *cis*-elements are potentially responsive to abscisic acid and salicylic acid. The PlantCARE and PLACE tools revealed the presence of the CATTTG motif, which is the binding site for the MYC transcription element and has a role in jasmonic and abscisic acid signaling. The promoter contains an anaerobic induction element (ARE), a wound-responsive element (motif), and a stress-responsive element (TC-rich repeats), suggesting that the *PvH_HMGR* gene may be involved in various stress responses in Vietnamese ginseng. Besides, CpG sequence clusters have not been detected in the predicted promoter region, suggesting a potential lack of inhibition of gene expression due to cytosine methylation.

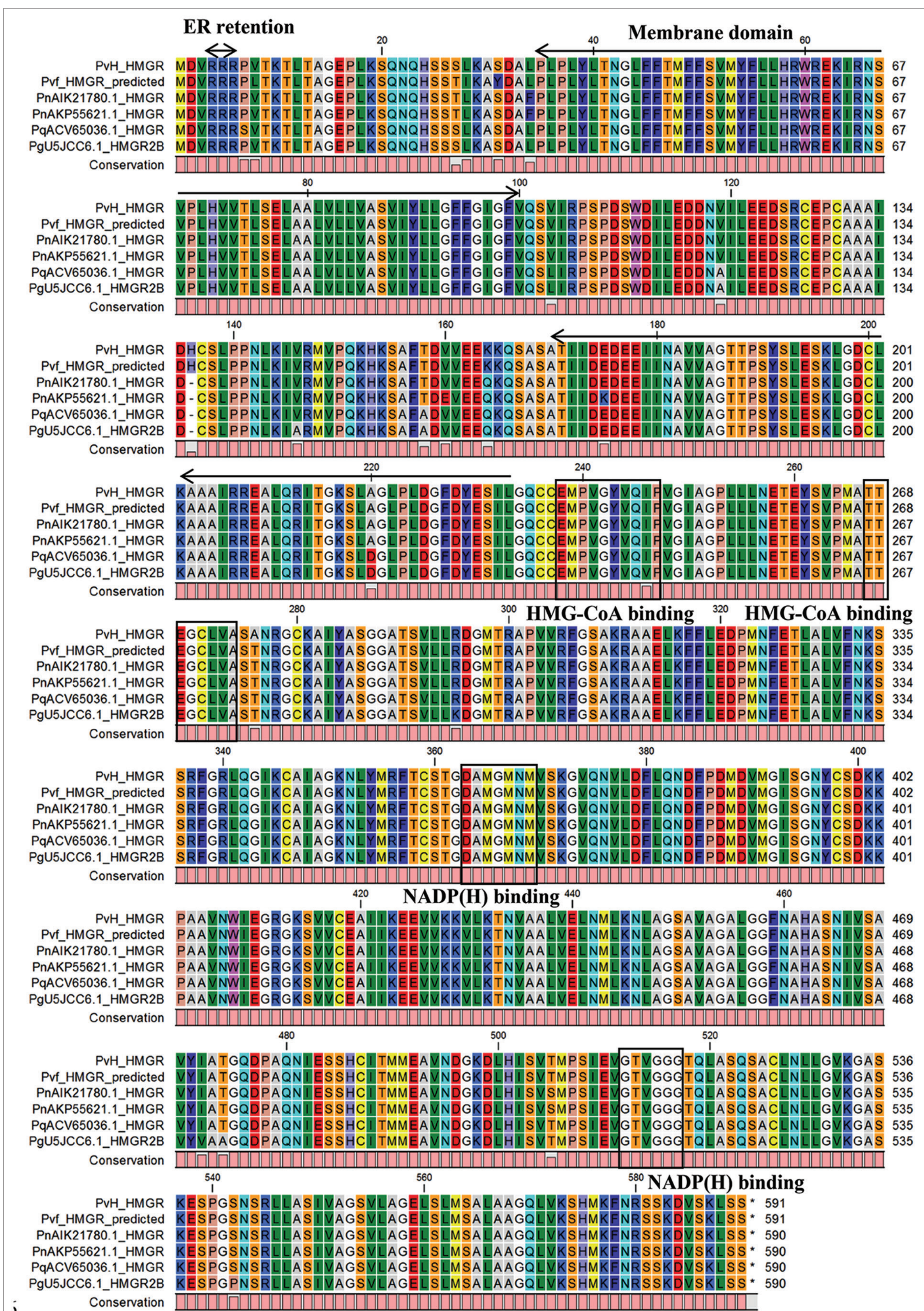

Figure 1: Multiple alignments of PvH_HMGR deduced amino acid sequences with *Panax* genus homologous HMGRs. *P. vietnamensis* var fucisous (PvfHMGR), *P. quinquefolius* (ACV65036), *P. notoginseng* (AIK21780.1, AKP55621.1), and *P. ginseng* (U5JCC6.1). Two putative HMGR-CoA-binding sites and two NADP(h)-binding sites were indicated with a thin square box, and the motif responsible for ER retention was highlighted with a bold square box.

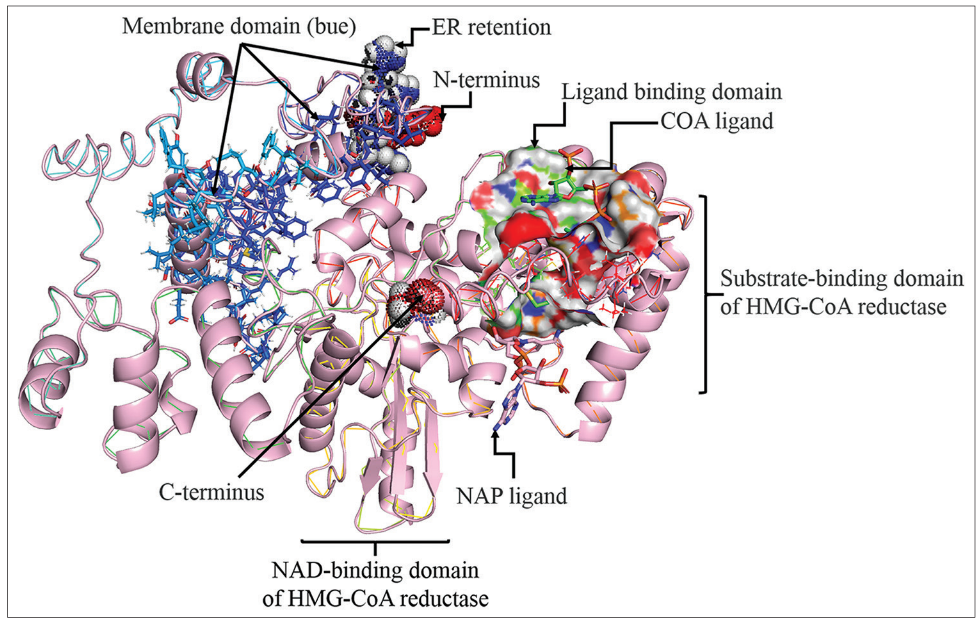

Figure 2: 3D structure prediction of PvH_HMGR with various annotations such as membrane domain (blue), ER retention, C-terminus, N-terminus, substrate-binding domain, beta-sheet, alpha-helix, and ligand binding domains (COA and NAD ligand).

Figure 3: Prediction of an important *cis*-regulatory element found in the promoter of *PvH_HMGR* gene. Red frame: translation initiation site, low letter DNA sequence: 5’ UTR, TSS: transcriptional start site, green frame: TATA-box, dark blue frame: ABRE *cis*-element involved in the response to abscisic acid, yellow frame: G -box *cis*-element involved in the response to light, dark green frame: TCA cis-element-probably involved in the response to salicylic acid, light green frame: TCT-motif involved in the response to light, purple frame: MYC motif.

3.5. Phylogenetic Analysis

The evolutionary relationships of the *PvH_HMGR* in Vietnamese ginseng with corresponding genes from some *Panax* species and various organisms (bacteria, archaea, fungi, plants, and animals) were aligned in MUSCLE and neighbor-joining trees generated by the MEGA X program with 1000 bootstraps [Figure 4]. The phylogenetic analysis results show that a large group of plant HMGRs originated from a more recent common ancestor with animal HMGRs than the other organisms. It may be suggested that the HMGR genes of the plant group diverged at a later period than the corresponding genes of groups such as archaea, bacteria, fungi, and animals. The HMGRs of the *Panax* genus formed a common cluster within the plant’s group and represented the most recently diverged lineage within this group. Specifically, the *PvH_HMGR* has a high similarity with corresponding HMGRs to *P. vietnamensis* var. fuscidiscus (99.7%), *P. notoginseng* (98.98%), *P. quinquefolius* (98.64%), and *P. ginseng* (97.80%).

3.6. Effect of Elicitors on the Expression of PvH_HMGR

The present study indicated that the addition of various concentrations of NaCl and Na_2CO_3 to create abiotic stress significantly improved the expression of the *PvH_HMGR* gene in adventitious roots of Vietnamese ginseng compared with the untreated control sample. In general, Na_2CO_3 had a stronger effect on the expression of PvH_HMGR than NaCl.

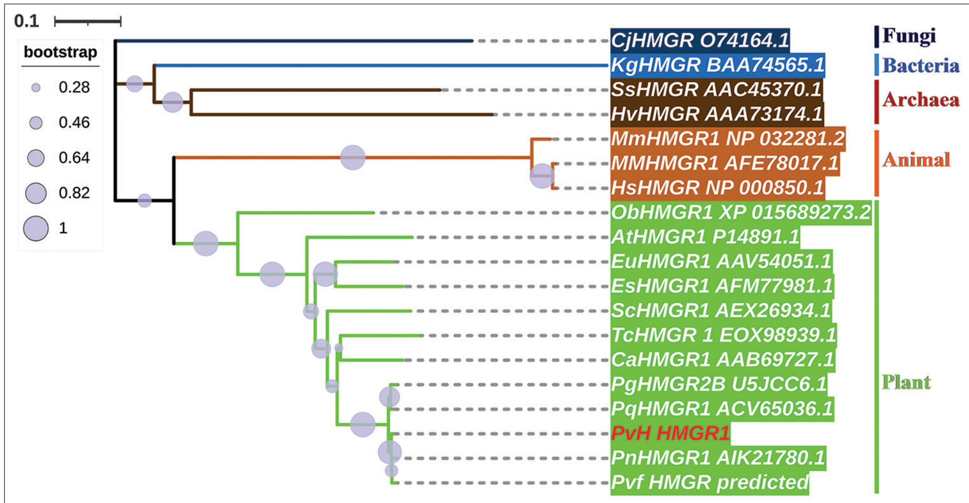

Figure 4: A phylogenetic tree of PvH_HMGR and various organisms. Plants (greenish-yellow: PvH: *P. vietnamensis*, Pvf: *P. vietnamensis* var fuscidiscus, Pg: *P. ginseng*, Pn: *P. notoginseng*, Pq: *P. quinquefolius*, Ca: *Camptotheca acuminata*, Sc: *Solanum chacoense*, Tc: *Theobroma cacao*, Es: *Eleutherococcus senticosus*, Eu: *Eucommia ulmoides*, At: *Arabidopsis thaliana*, Ob: *Oryza brachyantha*), animals (yellowish-orange: Hs: *Homo sapiens*, MM: *Macaca mulatta*, Mm: *Mus musculus*), fungi (light navy: Cj: *Cyberlindnera jadinii,* Pm: *Paraphaeosphaeria minitans*)*,* achaea (antique bronze: SS: *Saccharolobus solfataricus*, Hv: *Haloferax volcanii*), and bacterium (blue color: Kg: *Kitasatospora griseola*).

The semi-quantitative RT-PCR result showed that increasing the NaCl concentration up to 0.1 % increased the expression of *PvH_HMGR* about 2.71 times more than the untreated sample [Figure 5]. At this concentration, there is the little observable inhibitory effect on the growth of Vietnamese ginseng adventitious roots. This result is quite similar to the study [21] on *P. ginseng*, which added 0.1% NaCl to enhance the saponin content. In addition, Gupta's study [24] also showed that 0.1% NaCl increased the biosynthesis of steviol glycoside in *Stevia rebaudiana*. For the treatment with Na_2CO_3, the results showed the highest expression of *PvH_HMGR* at a concentration of 0.075%, reaching 3.28 times higher than that of the untreated control. Besides, Na_2CO_3 was found to inhibit adventitious root growth at a concentration higher than 0.05%. In general, for other tested concentrations of elicitors, the *PvH_HMGR* expression was reduced compared to the above ideal concentration.

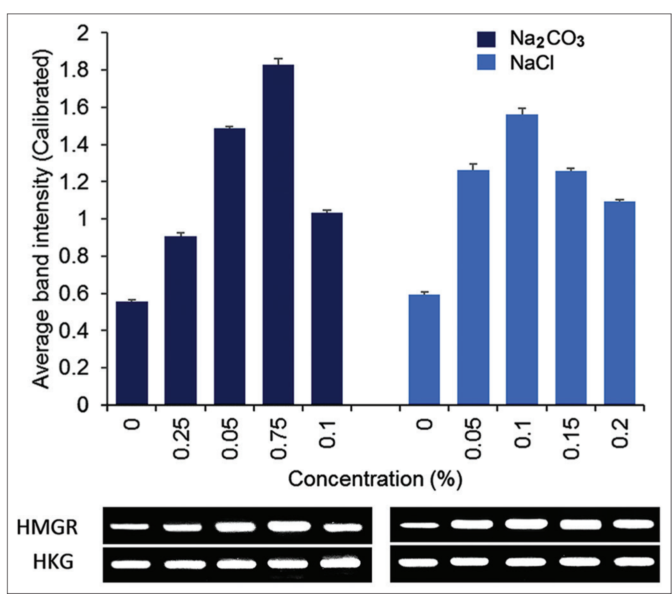

Figure 5: Semi-quantitative RT-PCR expression analysis of *HMGR* in adventitious root Vietnamese ginseng under untreated and treated NaCl and Na_2CO_3 at various concentrations. The *GAPDH* gene was used as the reference gene. The data on the chart were expressed as the mean of three replicates with standard error bars.

4. DISCUSSION

HMGR is a key regulatory enzyme with an important rate-limiting function in the ginsenoside and cholesterol biosynthesis pathways in plants, yeasts, and animals [27-29]. At present, HMGR genes have been cloned, characterized, and expression analyzed in the *Panax* genus, including *P. ginseng* [30], *P. quinquefolius* [31], *P. notoginseng* [32], and other plants such as *Centella asiatica* L. [33], *Sanghuangporus baumii* [34], and *Withania somnifera* [35]. Vietnamese ginseng is a precious ginseng that is proven to have a high content of ginsenoside, have anti-inflammatory effects, improve memory, be anti-stressing, and support anti-cancer [1,12]. However, at the present, there have not been any reports on HMGR genes in Vietnamese ginseng. This research showed the isolation of the cDNA sequences' structural and functional features and the identification of their promoter sequences in Vietnamese ginseng. The results indicated that PvH_HMGR is a functional ortholog of *P. vietnamensis* var. fuscidiscus and *P. ginseng* and that PvH_HMGR plays a similar regulatory role in the production of triterpene ginsenosides in ginseng plants. Besides, this study also shows that the PvH_HMGR feature of Vietnamese ginseng is similar to that of the active sites responsible for binding HMG-CoA and $NADPH_2$ among *Panax* species, including *P. ginseng*, *P. notoginseng*, and *P. quinquefolius*. The predicted homology modeling of PvH_HMGR, showing high similarity to human HMG-CoA reductase, provided important information on enzyme characterization for further studies of the enzyme's biological function and kinetics. The phylogenetic tree analysis showed that *PvH_HMGR* has more similarities with the corresponding genes from *P. vietnamensis* var. fuscidiscus *and P. notoginseng* than other species.

Plant cell biotechnology is considered a preferred solution for the production of large quantities of valuable compounds in a short

period because bioactive metabolites accumulate slowly in medicinal plants under natural conditions [36,37]. A better understanding of the key genes involved in secondary metabolism pathways, as well as the effects of elicitors on their activity, will allow for more effective biosynthesis of bioactive compounds using plant tissues and cell cultures [38]. The elicitors (chitosan, yeast extract, sodium chloride, sodium carbonate, methyl jasmonate, abscisic acid, or salicylic acid) have been studied to improve the expression of key genes related to plant secondary metabolism to enhance yields of secondary metabolite biosynthesis in plant cell cultures for a shorter time [21,24,39,40]. Continuing to investigate suitable and low-cost elicitors for improving high-value ginsenosides in Vietnamese ginseng in a short period of time is a necessary research direction. Up to now, there are no reports regarding the effect of salt (NaCl and Na_2CO_3) on the expression of key regulatory genes for saponin metabolism or ginsenoside production from root cultures of Vietnamese ginseng.

In comparison to other concentrations, 0.1% NaCl and 0.075% Na_2CO_3 increased the expression of the *HMGR* gene in Vietnamese ginseng roots. The previous studies indicated that this concentration of NaCl also increased the saponin content in *P. ginseng* and the steviol glycoside content in *S. rebaudiana* [21,24]. Nevertheless, the report of Gupta showed that sodium carbonate at a lesser concentration (0.025%) enhanced the terpenoid glycoside content in *S. rebaudiana* species [24]. This may imply that sodium carbonate may affect gene activity in terpenoid metabolism differently depending on the species.

The findings suggested that sodium chloride (0.1%) and sodium carbonate (0.075%) might be used as suitable abiotic elicitors to improve the production of ginsenoside in the adventitious root culture of Vietnam ginseng.

5. CONCLUSION

PvH_HMGR, the key enzyme in the ginsenoside production pathway of Vietnamese ginseng, was successfully cloned and identified. The characteristics and 3D structure of PvH_HMGR were predicted. Their core promoter was also predicted concerning various stress responses in Vietnamese ginseng. Furthermore, abiotic stress (NaCl and Na_2CO_3) significantly improved the expression of PvH_HMGR for ginsenoside synthesis in Vietnamese ginseng adventitious root culture.

6. AUTHOR CONTRIBUTIONS

Loc NH, Tien NQD, and Nhut DT designed and performed the overall study. Tien NQD, Man LQ, Kha H, and Linh TL conducted molecular cloning and bioinformatics analysis. Tien NQD, Kha H, Anh TL conducted elicitor treatment and analyzed gene expression. Nhut DT provides research samples. Tien NQD and Loc NH wrote the final manuscript.

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8. CONFLICTS OF INTEREST

All authors declared no conflicts of interest.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All data generated and analyzed are included within this research article.

11. PUBLISHER'S NOTE

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