

## Isolation and characterization of LHT-type plant amino acid transporter gene from *Panax ginseng* Meyer

Ru Zhang<sup>1,2</sup>, Jie Zhu<sup>1</sup>, Hong-Zhe Cao<sup>1</sup>, Xiao-Lei Xie<sup>1</sup>, Jing-Jia Huang<sup>1</sup>, Xiang-Hui Chen<sup>1</sup>, and Zhi-Yong Luo<sup>1\*</sup>

<sup>1</sup>Molecular Biology Research Center, School of Life Sciences, Central South University, Changsha 410078, China

<sup>2</sup>College of Chemistry and Chemical Engineering, Hunan Institute of Engineering, Xiangtan 411104, China

A lysine histidine transporter (LHT) cDNA was isolated and characterized from the roots of *Panax ginseng*, designated *PgLHT*. The cDNA is 1,865 bp with an open reading frame that codes for a protein with 449 amino acids and a calculated molecular mass of 50.6 kDa with a predicted isoelectric point of 8.87. Hydropathy analysis shows that *PgLHT* is an integral membrane protein with 9 putative membrane-spanning domains. Multiple sequence alignments show that *PgLHT* shares a high homology with other plant LHTs. The expression profile of the gene was investigated by real-time quantitative polymerase chain reaction during various chemical treatments. *PgLHT* was up-regulated in the presence of abscisic acid, salicylic acid, methyl jasmonate, NaCl, and amino acids. To further explore the function of *PgLHT* gene, full-length cDNA of *PgLHT* was introduced into *P. ginseng* by *Agrobacterium rhizogenes* A4. The overexpression of *PgLHT* in the hairy roots led to an obviously increase of biomass compared to the controls, and after addition of the amino acids, the overexpressed-*PgLHT* hairy roots grew more rapidly than untreated controls during early stage of the culture cycle. The results suggested that the *PgLHT* isolated from ginseng might have role in the environmental stresses and growth response.

**Keywords:** *Panax ginseng*, LHT gene, Abiotic stresses, Real-time quantitative polymerase chain reaction

### INTRODUCTION

Amino acids play central roles both as building blocks of proteins and as intermediates in metabolism. They are needed for cellular structure, growth, and development of plants. Amino acid uptake into cells and cellular compartments depends on membrane transporters and amino acid transporters have been identified in many organisms including bacteria, animals, and plants [1,2]. Amino acid transport is involved in all processes associated with resource allocation during plant growth and development [3,4]. Therefore, identifying various amino acid

transporter genes that participate in resource allocation is essential for understanding this fundamental process.

Amino acid transporters in higher plants are classified into two superfamilies: the amino acid transporter (ATF) and the amino acid-polyamine-choline facilitator (APC) [2,4]. Members of the ATF superfamily have been shown to have 9 to 11 membrane-spanning domains, whereas members of the APC superfamily contain 12 or 14 putative transmembrane domains [5,6]. The ATF superfamily contains the amino acid permease (AAP), lysine- and

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Edited by Tae-Jin Yang, Seoul National University, Korea

Received 03 Dec. 2012, Revised 29 Jan. 2013, Accepted 04 Mar. 2013

\*Corresponding author

E-mail: [luo\\_zhiyong@hotmail.com](mailto:luo_zhiyong@hotmail.com)

Tel: +86-731-84805025, Fax: +86-731-84484492

histidine-specific transporter (LHT), proline transporter,  $\gamma$ -aminobutyric acid transporter, aromatic and neutral amino acid transporter, and auxin transporter [3].

AAP1 was the first isolated by functional complementation [7,8] and it was subsequently shown to be part of a small gene family of related transporters that are differentiated by substrate specificity and expression patterns [9,10]. Since then many AAPs were separated and all Arabidopsis AAPs (*AtAAPs*) have been identified as H<sup>+</sup>-coupled amino acid uptake systems [9,11]. AAPs have been suggested to be involved in a number of physiological processes in plants including amino acid uptake from the soil [12].

However, much less is known about the LHTs, a family of 10 members was found in Arabidopsis. *AtLHT1* was originally described as a lysine and histidine selective transporter [6], but other studies with *AtLHT1* and *AtLHT2* suggest that LHTs preferentially transport neutral and acidic amino acids with high affinity [11,13]. Although *AtLHT1* was previously reported to be a broad-spectrum amino acid transporter [11], these abundant amino acids are possibly the main physiological substrates of *AtLHT1*. In recent investigations demonstrated that *AtLHT1* is a negative modulator of disease resistance and that its substrate, Gln, when integrated with the SA pathway, plays pivotal roles in plant defense responses [14]. In this study, a putative LHT gene was isolated and characterized from the roots of *Panax ginseng*. Sequence comparison showed that this ginseng gene may belong to the LHT subfamily and may be a plasma membrane-located protein, as indicated by the existence of its 9 transmembrane helices. Isolation and functional analysis of the *PgLHT* gene from *P. ginseng* will help to improve our knowledge of amino acid metabolism and accumulation in this important medicinal plant.

## MATERIALS AND METHODS

### Plant materials

Ginseng (*P. ginseng* Meyer) was collected from Fusong County, Jinlin Province, China. Four-year-old fresh ginseng roots were used for gene cloning and hairy roots induction. Ginseng stems, roots, leaves, and hairy roots were used for gene expression analysis.

### LHT gene cloning and sequence analysis

Total RNA was isolated from roots of 4-year-old ginseng plants. cDNA was synthesized using BD SMART RACE cDNA Amplification Kit (Clontech, Palo Alto, CA, USA) according to the manufacturer's protocol. All

polymerase chain reaction (PCR) products were cloned into the pGEM-T-Easy vector (Promega, Madison, WI, USA) and sequenced. To amplify the core fragments, a 729 bp fragment cDNA was amplified using two degenerate primers: 5'-TGGCAAATGGTKGADATGCATGA-3' as forward primer, 5'-WATCTGGTADCTWCCDATDACATG-3' as reverse primer. PCR reaction was performed in a 50  $\mu$ L reaction volume containing 5  $\mu$ L of 10 $\times$ Ex Taq Buffer (Mg<sup>2+</sup> Plus), 0.5  $\mu$ L of dNTP (10 mM), 1  $\mu$ L of each primer (10  $\mu$ M), 41.25  $\mu$ L of ddH<sub>2</sub>O, 0.25  $\mu$ L (1 U) of Ex Taq polymerase (Takara, Ohtsu, Japan), and 1  $\mu$ L of cDNA template. The PCR conditions were: 94°C for 30 s, 65°C for 30 s, 72°C for 60 s (10 cycles, scaledown of 1°C per cycle); 94°C for 30 s, 56°C for 60 s, 72°C for 60 s (25 cycles); 72°C for 10 min.

To isolate the LHT full-length cDNA of *P. ginseng*, 5' RACE and 3' RACE were carried out using the SMART RACE amplification kit (Clontech) with 5' RACE primer (5'-CTCGGGTGTGGAAGGGATTGTTGC-3') and 3' RACE primer (5'-GTCCACTTTGTCCTCTCCCATCTCC-3') respectively. PCR reaction systems were used in the kit-recommended 50  $\mu$ L PCR reaction. The PCR conditions were: 94°C for 4 min; 94°C for 30 s, 72°C for 90 s, 5 cycles; 94°C for 30 s, 70°C for 30 s, 72°C for 2 min, 5 cycles; 94°C for 30 s, 68°C for 30 s, 72°C for 2 min, 20 cycles.

The open reading frame (ORF) was analyzed using an ORF finder tool from the web (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>). Multiple sequence alignments were performed using BLAST and ClustalX program [15,16]. Phylogenetic analysis of deduced amino acid alignments was performed using the neighbor-joining method with the MEGA 4.1 program [17]. Bootstrap analysis with 1,000 replicates was used to assess the strength of nodes in the tree. Prediction of transmembrane domains was generated using the TMHMM 2.0 (<http://www.cbs.dtu.dk/services/TMHMM-2.0>) and TMPred server ([http://www.ch.embnet.org/software/TMPRED\\_form.html](http://www.ch.embnet.org/software/TMPRED_form.html)).

### Chemical treatment of hairy roots

To investigate the response of *PgLHT* gene to various chemical treatments, two weeks cultured hairy roots (transformed by *Agrobacterium rhizogenes* A4 without any foreign DNA) were used. For chemical treatment, hairy roots were maintained in Murashige and Skoog (MS) media containing indicated concentrations of chemicals; 20  $\mu$ M abscisic acid (ABA), 200  $\mu$ M salicylic acid (SA), 100  $\mu$ M methyl jasmonate (MeJA), 50 mM NaCl, and 36  $\mu$ M L-amino acids (Thr, Ser, Asn, Glu, Gln, Pro, Gly, Ala, Val, Cys, Met, Leu, Tyr, Phe, Lys,

His, Arg, Asp 2  $\mu\text{M}$  each). The hairy root samples were collected after post-treatment. The treated hairy roots were immediately frozen in liquid nitrogen and stored at  $-70^\circ\text{C}$  until required.

### Real-time quantitative polymerase chain reaction analysis

The gene transcript levels of different tissues and hairy roots of ginseng were quantified by real-time quantitative PCR (qRT-PCR) using the SYBR Green stains on the Mastercycler ep realplex2 detection system (Eppendorf, Hamburg, Germany). Total RNA was extracted from ginseng samples using EZNA Plant RNA kit (Omega Biotek, Doraville, GA, USA) according to the manufacturer's instructions. Reverse transcription reactions were performed using RevertAid First Strand cDNA Synthesis kit (Fermentas, Hanover, MD, USA) according to the supplier's manual. A 25- $\mu\text{L}$  reaction mixture contained 12.5  $\mu\text{L}$  of 2 $\times$ SYBR Premix Ex Taq II (Takara), 1  $\mu\text{L}$  each of 10  $\mu\text{M}$  forward and reverse primer and a suitable amount of cDNA. The PCR conditions were:  $95^\circ\text{C}$  for 30 s; 40 cycles of  $95^\circ\text{C}$  for 10 s,  $60^\circ\text{C}$  for 30 s. At the end of the amplification cycle, a melting analysis was conducted to verify the specificity of the reaction. This was carried out by heating the amplification products from  $60^\circ\text{C}$  to  $95^\circ\text{C}$  at  $0.5^\circ\text{C}/10$  s. Post qRT-PCR calculations to analyze relative gene expression were performed according to the  $2^{-\Delta\Delta\text{Ct}}$  method as described [18]. The data for the genes expression are shown after normalization with the  $\beta$ -actin internal control (GenBank accession no. AY907207). All qRT-PCR reactions were performed in triplicate. Specific primers for *PgLHT* were 5'-CTGGGAGATGTAGCGTTTG C-3' as forward, 5'-GGTGTTGAAGGGATTGTTGC-3' as reverse; and  $\beta$ -actin were 5'-TGC CCCAGAAGAGCACCTGT-3' as forward, 5'-AGCATACAGGGAAAGATCGGCTTGA-3' as reverse.

### Vector construction and plant transformation

The *PgLHT* cDNA was amplified by specific primers (5'-CGGGATCCCGCACCATGG TTTCCCAAGCTCAGAC-3' as forward and 5'-CGCGAGCTCGGCTCCTTTGCTGCTTAT GAGAA-3' as reverse) from *P. ginseng* and was digested with *Bam*H I and *Sac* I to yield a 1,350 bp fragment containing the complete open reading frame. This fragment was then ligated into the same restriction site of pBI121 vector which contains a CaMV35S promoter, a nopaline synthase (NOS) terminator, and a kanamycin resistance marker. The recombinant binary vector pBI121 was subsequently confirmed

by DNA sequence analysis. The pBI121 vector harboring *PgLHT* construct was introduced into *A. rhizogenes* A4 by a conventional method [19].

The 4-year-old fresh ginseng roots were used to induce hairy roots. Ginseng roots were sterilized and cultured as previously described methods [20]. Sterilized roots were sliced into 3-5 mm-thick discs. Root discs were wounded with a sterilized needle loaded with *A. rhizogenes* A4 containing pBI121 binary vector. After 3 days, the root discs were transferred onto the MS medium containing 100 mg/L kanamycin and 400 mg/L cefotaxime for selection of transformed roots. After cultivation of 30 to 50 days, the hairy roots were picked out and transferred on fresh medium with cefotaxime and kanamycin for several generations until the bacteria were eliminated. Hairy roots were transformed by *A. rhizogenes* A4 harboring empty pBI121 vector used as controls.

### Analysis of transgenic hairy roots

RT-PCR and qRT-PCR were done to determine the transgenic hairy roots. RNA extraction and reverse transcription reaction were performed by following the method used as before. In the RT-PCR, specific primers for *PgLHT* were (5'-GCCTCTGTCCACTTTG TCCTCT-3') as forward and (5'-TCTCGGGTGTGAAGGGATTGT-3') as reverse; and  $\beta$ -actin (GenBank accession no. AY907207) were 5'-CGTGATCTTACAGATAGCTTCA TGA-3' as forward, 5'-AG AGAAGCTAAGATTGATCCTCC-3' as reverse. PCR reaction was performed in a 50  $\mu\text{L}$  reaction volume containing 5  $\mu\text{L}$  of 10 $\times$ Taq buffer ( $\text{Mg}^{2+}$  free), 3  $\mu\text{L}$  of  $\text{MgCl}_2$  (25 mM), 4  $\mu\text{L}$  of dNTP (2.5 mM each), 2  $\mu\text{L}$  of each primer (10  $\mu\text{M}$ ), 32.75  $\mu\text{L}$  of ddH<sub>2</sub>O, 0.25  $\mu\text{L}$  (1 U) of Taq polymerase (Takara) and 1  $\mu\text{L}$  of cDNA template. The amplifications were carried out under the following conditions:  $95^\circ\text{C}$  for 30 s; 30 cycles of  $95^\circ\text{C}$  for 30 s,  $58^\circ\text{C}$  for 30 s,  $72^\circ\text{C}$  for 40 s;  $72^\circ\text{C}$  for 10 min.

### Analysis of growth pattern

To evaluate the effects of overexpression of *PgLHT* on the growth of hairy roots, the overexpression-*PgLHT* lines and control lines were cultured in liquid MS medium with amino acids or not, harvested the hairy roots and the media was collected at the indicated time respectively. The harvested hairy roots were rinsed with sterile dH<sub>2</sub>O, dried on filter paper and weighed securely. The media were filtered and amino acids of the media were assayed using the L-amino acid quantitation kit (BioVision, Milpitas, CA, USA) according to the manufacturer's instructions. A total 50  $\mu\text{L}$  reaction mix containing:

46 µL L-amino acid assay buffer, 2 µL L-amino acid probe, 2 µL L-amino acid enzyme mix. And then 50 µL of the reaction mix was added to each well containing the test samples, incubated 30 minutes at 37°C and assayed at 570 nm. All samples were corrected background by subtracting the value of the 0 µM amino acid controls. Amino acid concentration was calculated according to the formula of the manufacturer's instructions.

## RESULTS AND DISCUSSION

### Isolation and amino acid sequence analysis of *PgLHT*

In order to isolate full-length of ginseng LHT gene, a pair of degenerative primers was designed according to the most conservative region of a known lys- and his-specific amino acid transporter from Arabidopsis and other plants. A 729 bp fragment was cloned successfully. 3'RACE and 5'RACE were carried out using the corresponding gene specific primers designed in the light of the fragment which was cloned by degenerate primers. This gene is named *PgLHT* with a GenBank accession number JX896444. The *PgLHT* cDNA is 1,865 base pairs (bp) long and have 5'-UTR (129 bp) and 3'-UTR (386 bp) sequences at both ends. The ORF encodes for a protein of 449 amino acids with a predicted molecular weight of 50.6 kDa with a predicted isoelectric point of 8.87.

The model for the location of the trans-membrane helices predicted by TMHMM 2.0 is shown in Fig. 1A. Hydropathy analysis shows that *PgLHT* is an integral membrane protein with 9 putative membrane-spanning domains, and amino acid residues are located at 41-63, 68-90, 161-178, 183-205, 271-293, 313-335, 356-378, 382-404, and 417-439 respectively. We also analyzed the structure by using TMpred software which revealed also the possible existence of 9 membrane-spanning regions, located at 44-63, 66-87, 161-177, 183-202, 221-242, 270-290, 309-327, 361-380, and 417-435, respectively, the putative membrane-spanning regions are marked by arrows (Fig. 1B). These domains are consistent with its plant LHT family [2].

### Homology analysis

An amino acid sequence comparison using BLASTP revealed the high degree of similarity between the predicted ginseng LHT transporter and other LHT transporters from plants. The alignment using ClustalX is shown in Fig. 2A. Sequence analysis showed high identities with other species such as *Glycine max* (*GmLHT*,

XM003547627, 83.41% identity), *Arabidopsis thaliana* (*AtLHT1*, NM180778, 79.65% identity), *Lotus japonicus* (*LjLHT*, JF705953, 72.46% identity), *Populus trichocarpa* (XM002315593, 69.23% identity). Phylogenetic analysis was performed based on LHTs amino acid sequences with the MEGA 4.1 program. *PgLHT* shares the highest homology with the LHT from *G. max* (Fig. 2B).

### Tissue-specific expression patterns of *PgLHT*

The qRT-PCR analysis was performed to investigate the patterns of *PgLHT* gene expression in different tissues of *P. ginseng*. As illustrated in Fig. 3, the *PgLHT* transcript was constitutive expressed in roots, stems, leaves, and hairy roots, the highest level was found in leaves, significantly lower expression was found in stems. In Arabidopsis, amino acid transporter genes have also various expression patterns in different tissues such as *AAP3* is exclusively expressed in roots and *AAP4* mainly in leaves, stems, and flowers whereas *AAP5* is found in all tissues. *ProT1* was found to be expressed in all organs of Arabidopsis, but highest levels were found in roots, stems, and flowers [21].

### Expression profiles of *PgLHT* in response to chemical treatments

Hormone-induced plant growth regulators like ABA, SA, or jasmonic acid (JA) differentially activate stressed-related genes expression [22]. As shown in Fig. 4, *PgLHT* was upregulated at different degree to various hormone treatments. Under ABA treatment, the *PgLHT* expression increased steadily and gradually reached a higher level at 24 h (5.5-fold), which can be explained by the role of ABA. Recent studies suggested that ABA-mediated signaling also plays an important part in plant responses to environmental stresses and plant pathogens [23].

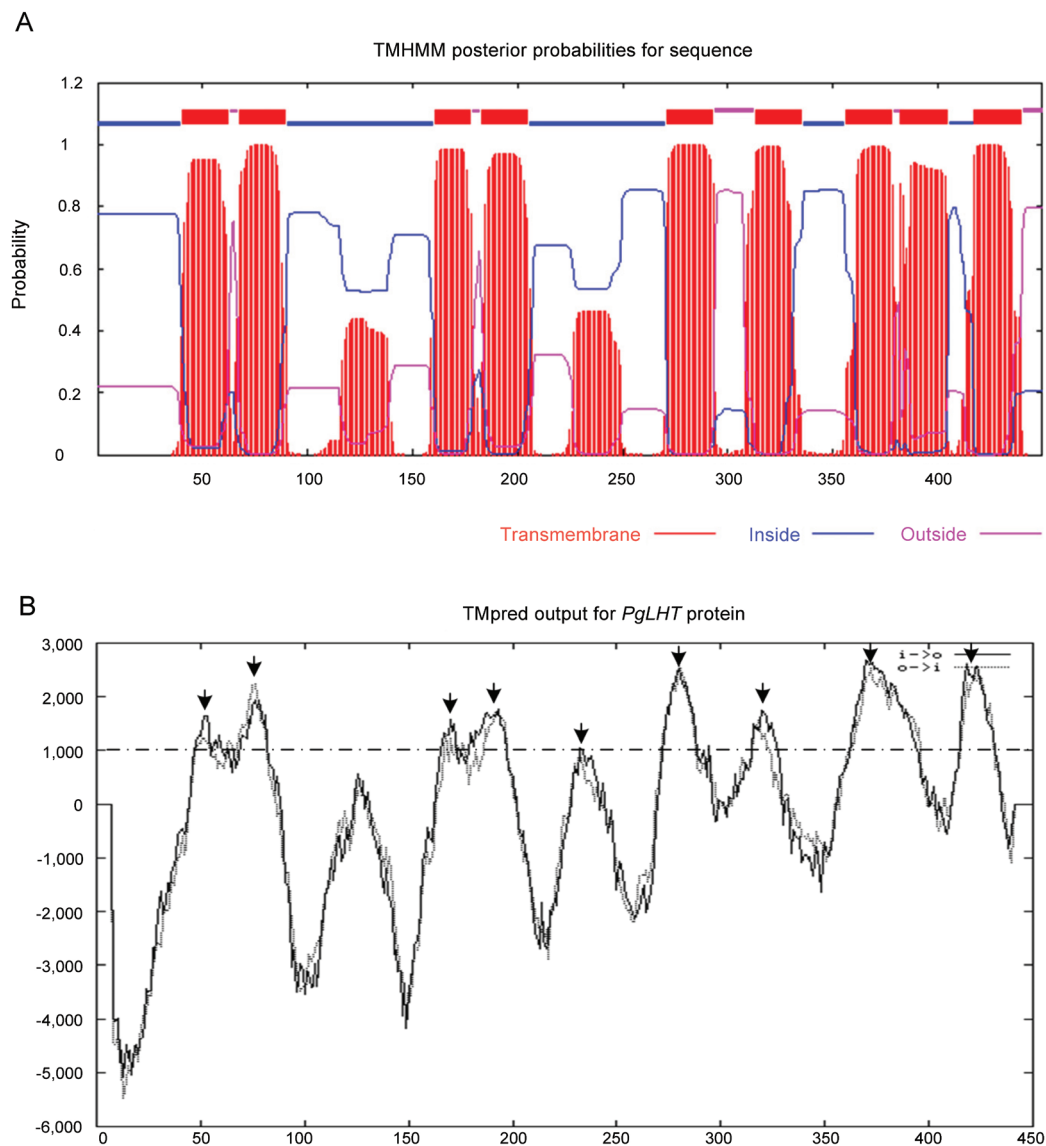
In the case of SA treatment, *PgLHT* expression maintained a lower level and reached higher point at 12 h with 3.14-fold and then sustained as steady level. *AtLHT1*, an important amino acid transporter, initially defined as a lys- and his-specific transporter [6], was later reported to transport a broad spectrum of amino acids in a heterologous yeast expression system [12]. It was also observed that *AtLHT1* expression was induced within 1 h of SA treatment. It is demonstrated that *AtLHT1* negatively modulates defense responses by interacting with the SA-dependent pathway [14]. The results imply that *PgLHT* may be mediating the defense responses in SA-dependent way in ginseng.

*PgLHT* expression level increased immediately after

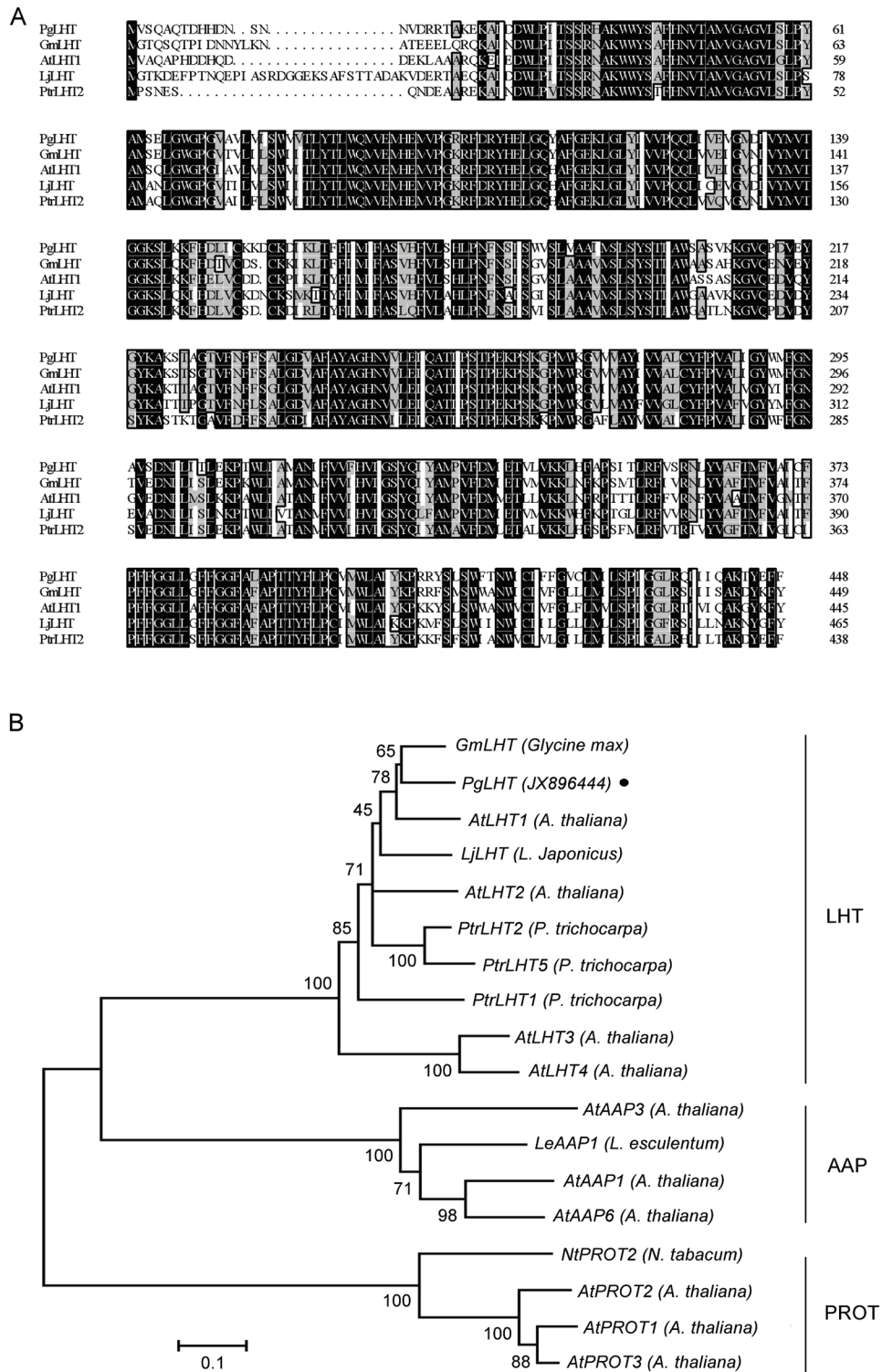
MeJA treatment and increased slowly until 12 h and reached 26.05-fold at 24 h. As well-known, MeJA is a lipid-based hormone signals that regulate a wide range of processes in plants, ranging from controlling plant insect defense, wounding responses, development to secondary metabolite synthesis [24]. In general, genes that were markedly up-regulated by JA or MeJA, were not SA-induced and vice versa, consistent with the known antagonistic effect of JA/MeJA and SA on gene expression [25]. As an exception to this rule, *PgLHT* was up-regulated by MeJA as well as SA.

At a salinity stress, *PgLHT* expression level increased

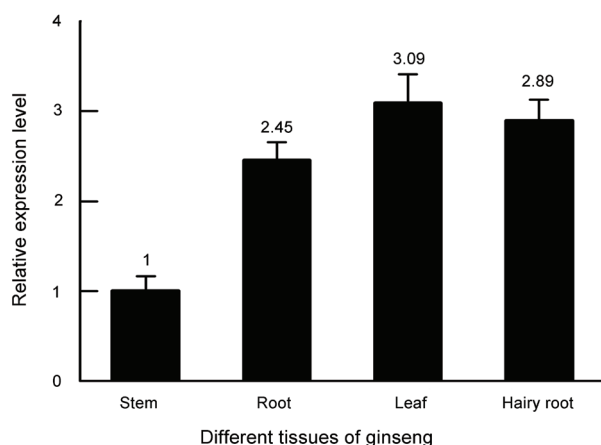
gradually to 10.27-fold at 12 h and then decreased rapidly at 24 h. High salinity reduces plant growth by affecting the plant's osmotic or ionic homeostasis [26]. Salinity stress in soil or water is one of the major abiotic stresses especially in arid and semi-arid regions and can severely limit plant growth and yield. *ProT1* and *ProT2* are widely expressed proline-specific amino acid transporters in *Arabidopsis*, they respond differentially to changes in water and salinity stress. *ProT2* expression was strongly induced under water or salinity stress [21], implying that *PgLHT* are similar to *ProT2* and play an important role in nitrogen distribution during water stress.



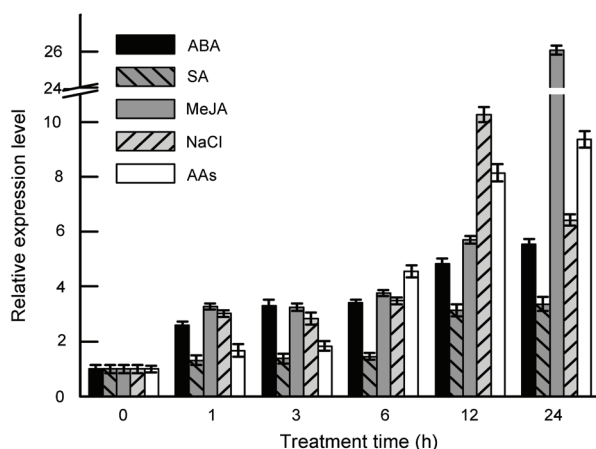
**Fig. 1.** Results of transmembrane helices prediction by TMHMM and TMPred. (A) The result of transmembrane helices prediction by TMHMM Server ver. 2.0. Red bars indicate transmembrane domains, blue lines indicate intracellular loops and magenta lines indicate extracellular loops. (B) Hydrophobicity plot of *PgLHT* obtained using TMPred. The ordinate represents the TMPred score divided by 1,000. Positive scores suggest hydrophobic region. The putative membrane-spanning regions are marked by arrows.



**Fig. 2.** Sequence homology and phylogenetic analysis of *PgLHT* compared to other lysine histidine transporter (LHT) proteins. (A) Comparison of the putative amino acid sequences of *PgLHT* and those of LHT genes from the following plants: *Glycine max* (GmLHT, XM003547627), *Arabidopsis thaliana* (AtLHT1, NM180778), *Lotus japonicus* (LjLHT, JF705953), *Populus trichocarpa* (PtrLHT2, XM002315593). (B) Phylogenetic analysis of the amino acid sequences of *PgLHT* and the other plant LHTs. Phylogenetic tree of plant LHTs distances between each clone and group are calculated with the program Mega 4.1 using the neighbor-joining method. The scale bar (number of substitutions/site) corresponds to the relative branch length, where a scale of 0.1 represents 10% change. AAP, amino acid permease; PROT, proline transporter.



**Fig. 3.** Relative mRNA expression levels of *PgLHT* in different tissues of ginseng. The error bars represent the standard error of means of three independent replicates.

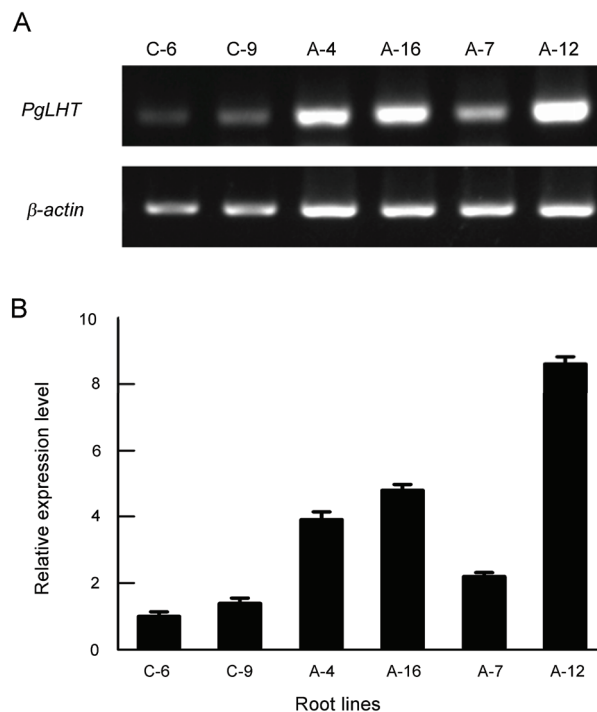


**Fig. 4.** Relative mRNA expression levels of *PgLHT* at various time points after chemical treatments. Abscisic acid (ABA), 20  $\mu$ M; salicylic acid (SA), 200  $\mu$ M; methyl jasmonate (MeJA), 100  $\mu$ M; NaCl, 50 mM; amino acids (AAs), 36  $\mu$ M. The error bars represent the standard error of means of three independent replicates.

A mixture of 18 amino acids was added to the culture of the hairy roots to investigate the expression pattern of *PgLHT*. After addition the amino acids, *PgLHT* expression increased steadily to a higher level (9.36 fold) at 24 h. Recent studies of *Arabidopsis* have identified several transporters as being important for amino acid uptake, and the transporters are regulated by its substrates of amino acids [27], *PgLHT* was upregulated in a similar way after addition the amino acids. Therefore, there may be a strong relationship between *PgLHT* expression and uptake of amino acid in ginseng.

#### Overexpression analysis of *PgLHT* in ginseng hairy roots

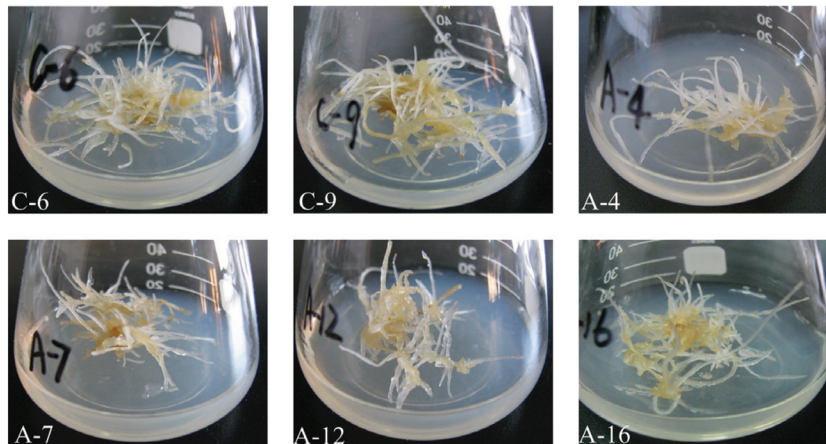
*P. ginseng* root was transformed using *A. rhizogenes*



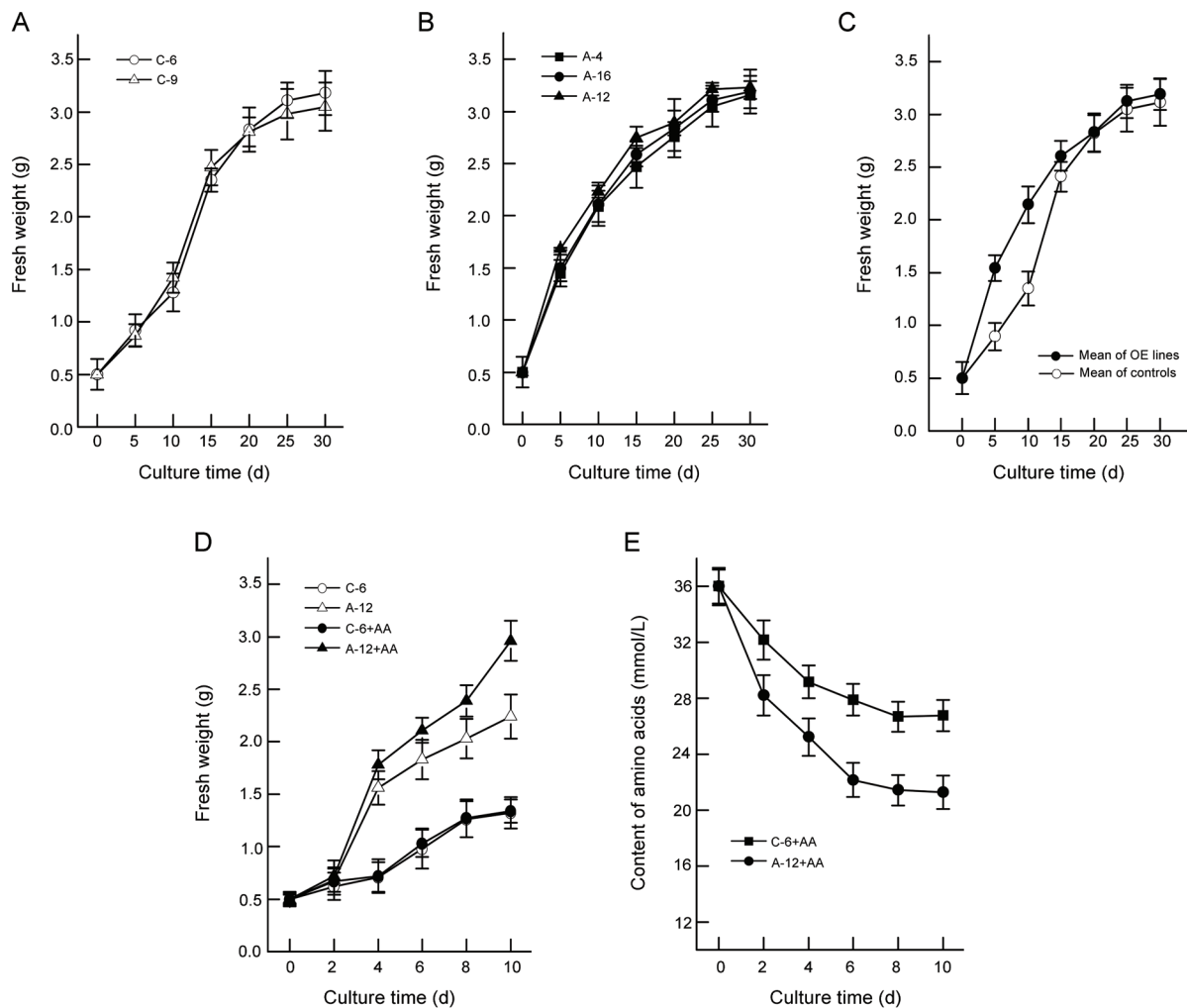
**Fig. 5.** Real-time (RT)- polymerase chain reaction (PCR) and real-time quantitative (qRT)-PCR analysis of *PgLHT* transcripts in control (empty vector; C-6 and C-9) and overexpression-*PgLHT* (A-4, A-7, A-12, and A-16) hairy root lines harvested at 15 day after subculture of the culture cycle. (A) RT-PCR. (B) qRT-PCR. The error bars represent the standard error of means of three independent replicates.

A4 with the *PgLHT*. Several independent transgenic lines were established by selection on kanamycin. Kanamycin resistant hairy roots were confirmed by RT-PCR and qRT-PCR analysis (Fig. 5). The expression levels of all overexpression-*PgLHT* lines tested, except A-7, are much higher than the average of controls (transformed with empty vector) (Fig. 5). The transformed hairy roots were indicated in Fig. 6. Two control hairy root lines and four overexpression-*PgLHT* lines were adapted to liquid MS medium with 5% sucrose and maintained for more than six generations before further analysis. The hairy root lines harvested midway through a culture cycle of 4 wk were subjected to qRT-PCR analysis again. The stable overexpression-*PgLHT* lines (A-12, A-16) were obtained for the further investigation.

In liquid MS medium, the overexpression-*PgLHT* lines showed a faster growth than control lines until 15 d after subculture, as determined by Student's *t*-test ( $p < 0.05$ ) between the average of overexpression-*PgLHT* lines and control lines, but there was no obvious difference in the final biomass (Fig. 7A-C). This implied that overexpression-*PgLHT* had positive effects on hairy root growth during early days of the culture cycle. To further



**Fig. 6.** Hairy roots induced from roots of *Panax ginseng*. Hairy roots of controls (C-6 and C-9) and overexpression-*PgLHT* lines (A-4, A-7, A12, and A-16) cultivated on solid Murashige and Skoog medium at 26°C at 15 d after subculture of the culture cycle. All lines presented the typical traits of hairy roots and had no morphological difference.



**Fig. 7.** Growth curves (A-D) and amino acids analysis (E) of hairy root lines. (A) Control lines. (B) Overexpression-*PgLHT* lines. (C) Average of controls and overexpression-*PgLHT* (OE) lines. (D) The growth curves of control (C-6) and overexpression-*PgLHT* line (A-12) after addition of the amino acids (AAs). (E) The amino acids content in the media of control (C-6) and overexpression-*PgLHT* line (A-12) after addition of amino acids (36  $\mu$ M, AAs). The error bars represent the standard error of means of three independent replicates. The initial inoculum for all lines weighed 0.5 g.



analyze the effect on the initial stage of overexpression-*PgLHT* line, the growth curves until 10 days after subculture were studied. As shown in Fig. 7D, there was an obvious increase of overexpression-*PgLHT* line (A-12) than control line (C-6) and biomass of overexpression-*PgLHT* hairy root line increased with the addition of amino acids (Fig. 7D, A-12+AA) in a time-dependent manner up to 2.96 g fresh weigh at 10 d, there was a 1.32-fold increase from the values without amino acids (A-12). Both overexpression-*PgLHT* line and control line caused amino acids content to a obvious decrease (2 to 6 d after subculture) in the media after exogenous addition of amino acids, but its was maintained at a steady level from 8 d after subculture. Amino acids content in overexpression-*PgLHT* lines are obviously lower than its control lines (Fig. 7E). Comparison of Fig. 7A-D, we presume that *PgLHT* may participate in the uptake of amino acids or other nutrients from the media.

In this study, overexpression-*PgLHT* lines of *P. ginseng* did not show any morphological differences (Fig. 6) when compared to controls. However, overexpression of *PgLHT* led to rapid growth of these hairy roots during early stage of the culture cycle. In *Arabidopsis* plants, LHT1 is crucial for Gln uptake at low concentrations and the magnitude of the increase in the uptake rate for Gln was similar to the magnitude of the increase in the growth response of plants overexpressing LHT1[27]. Hence, we can speculate that *PgLHT* plays an important role between amino acid uptake and growth of hairy roots in the initial culture.

In summary, we isolated *PgLHT* gene from ginseng roots and characterized its expression patterns in response to various chemicals. The accumulation of *PgLHT* transcripts was obviously induced after treatment with ABA, SA, MeJA, NaCl, and amino acids. The growth of the hairy roots was correlated with the overexpression-*PgLHT* which may mediate the amino acids or other nutrients uptake. The physiological function of the *PgLHT* is still needed to further study.

## ACKNOWLEDGEMENTS

The study was supported by the National Natural Science Foundation of China (grant no. 81071821, 81250110086, and 81101655), the Scientific Research Starting Foundation for Returned Overseas Chinese Scholars, Ministry of Education, China (grant no. 311101013), the Hunan Provincial Natural Science Foundation (grant no.12JJ3099), and the Scientific Research Fund of Hunan Provincial Education Depart-

ment (11C0329). The critical reading of the manuscript by Mohammed Nuruzzaman and Sadia Afrin, Research Fellow, Molecular Biology Research Center, School of Biological Science and Technology, Central South University is greatly appreciated.

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