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甘薯非特异性脂质转移蛋白基因克隆与表达分析

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摘 要: 非特异脂质转移蛋白(nsLTP)是植物界普遍存在的一类涉及多种胁迫反应的可溶性蛋白。为了阐明甘薯中非特异性脂质转移蛋白基因 *IbLTP1* 和 *IbLTP2* 在盐胁迫反应中的功能, 本研究运用 PCR 技术, 对 *IbLTP1* 和 *IbLTP2* 基因进行了克隆, 并通过生物信息学方法分析了序列结构、蛋白质保守结构域和系统进化关系; 利用 qRT-PCR 方法检测了这两个基因在不同组织中的表达模式以及盐胁迫条件下的表达差异; 将 *IbLTP1* 和 *IbLTP2* 基因克隆到大肠杆菌的原核表达载体 pET32a 中, 对重组菌 BL21(pET32a-LTP) 的耐盐性进行分析。序列分析表明: *IbLTP1* 和 *IbLTP2* 编码区均不含内含子并都具有等位基因。*IbLTP1* 和 *IbLTP2* 基因的蛋白质序列分别包括 114 和 94 个氨基酸残基并且不含色氨酸残基, 蛋白序列 N 端含有信号肽序列。保守结构域和系统进化分析结果表明: IbLTP1 和 IbLTP2 均含有 nsLTP 蛋白的保守结构域, IbLTP1 属于 Type Ⅰ 而 IbLTP2 属于 Type Ⅱ。实时荧光定量 PCR 分析表明: *IbLTP1* 在幼叶中表达量最高, 根中表达量最低; 而 *IbLTP2* 在茎中表达量最高, 成熟叶中表达量最低。在 NaCl 胁迫条件下, *IbLTP1* 和 *IbLTP2* 表达量在根中基本无变化而在茎和叶中上调。大肠杆菌 BL21(DE3)中异源表达 *IbLTP1* 和 *IbLTP2* 基因能够提高转基因菌株对 NaCl 的耐受性。因此, 本研究推测 *IbLTP1* 和 *IbLTP2* 基因可能在甘薯盐胁迫反应中发挥了作用。

关键词: 甘薯; 非特异性脂质转移蛋白; 氯化钠胁迫; 基因克隆; 基因表达; 实时荧光定量 PCR
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Isolation of Two Genes Encoding Nonspecific Lipid Transfer Protein and Their Expression Profiles in *Ipomoea batatas*

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Abstract: Nonspecific lipid transfer proteins (nsLTPs) are widely distributed in the plant kingdom and are involved in various stress responses. To clarify the function of *nsLTP* genes, *IbLTP1* and *IbLTP2* were cloned by PCR technology, and the sequence structures, conserved domains, and evolutionary relationships were analyzed. Sequences of cDNAs and genomic genes showed that neither gene had introns, but both had several homologous isoforms. *IbLTP1* and *IbLTP2* encode proteins of 114 and 94 amino acid residues respectively, without any Trp. These proteins contain a signal peptide at the N-terminal and have conserved domains of nsLTP1 and nsLTP2, respectively. The expression patterns and expression differences of *IbLTP1* and *IbLTP2* in different tissues and under stress were determined by

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real-time RT-PCR. Results showed that *IbLTP1* and *IbLTP2* had higher relative expression levels in young leaves and stems, respectively, and were highly induced under sodium chloride (NaCl) stress. The coding sequences of *IbLTP1* and *IbLTP2* were cloned into expression vector pET32a and expressed in *Escherichia coli* BL21 (DE3), respectively. The maximal OD_{600} values of strains harboring pET32a-IbLTP1 and pET32a-IbLTP2 were higher than those of the pET32a transformed strain under NaCl stress.

Key words: *Ipomoea batatas*; Nonspecific lipid transfer protein (nsLTP); Sodium chloride stress; Gene cloning; Gene expression; Real-time RT-PCR

Plants are constantly subjected to various abiotic stresses such as drought, flood, alkalinity, heavy metal toxicity, high salinity, and extreme temperatures throughout their life. In response to these abiotic stresses, plants often express numerous genes related to stress adaptation or tolerance to better adapt to the environments in which they grow.

Plant nonspecific lipid transfer proteins (nsLTPs) account for about 4% of total soluble protein content in higher plants[1], and are named due to their ability to promote the transfer of phospholipids between liposomes *in vitro* and broad substrate specificity. They are usually small, basic proteins, divided into two classes according to their molecular weights: that is 9 kD (Type Ⅰ)[2] and 7 kD (Type Ⅱ)[3]. All nsLTPs contain a signal peptide so that the mature protein can be transported to the outside of the cytoplasm. The signal peptides in Type Ⅰ and Type Ⅱ contain 21 – 27 and 27 – 35 amino acids, respectively [4]. Recently, however, additional types have been reported[5,6].

The nsLTP is comprised of four alpha-helices formed by eight conserved cysteine residues. The 3D structure of the nsLTP family in plants comprises a globular molecule stabilized by four disulfide bonds linking the helices to each other. Furthermore, nsLTPs, seed storage proteins, and trypsin-alpha amylase inhibitors have the same structural domains and superhelical, disulphide-stabilized four-helix bundle. The four disulfide bonds are formed by eight cysteine residues (8 CM), C-Xn-C-Xn-CC-Xn-CXC-Xn-C-Xn-C, where X is any amino acid and n is any number of amino acids. Based on the disulphide bridges, nsLTPs can be divided into two types. In Type Ⅰ, the cysteine residues 1 – 6, 2 – 3, 4 – 7, and 5 – 8 form disulphide bridges[7], whereas in Type Ⅱ, the cysteine residues 1 – 5, 2 – 3, 4 – 7, and 6 – 8 form disulphide bridges[8]. The cavities formed by hydrophobic amino acids for lipid binding are different between Type Ⅰ and Type Ⅱ. Type Ⅱ has lower lipid specificity, and the cavity of Type Ⅱ is more flexible than that of Type Ⅰ.

Lipids play an important role in plant signaling. Many hormones such as the phytosterols (derived from sterol) and jasmonates are considered lipids. These hormones are involved in different metabolic pathways, including defense response, programmed cell death, and germination. nsLTPs play an important role in plant responses to abiotic stresses[9] such as cold[10], drought[11], salinity[12] and oxidation[13], as well as biotic stresses such as bacterial and fungal pathogens[14]. The expression levels of *nsLTPs* often change when a plant is subjected to abiotic and biotic stresses. In addition, nsLTPs are related to surface cutin biosynthesis[15], signal transduction, and male sterility[16]. Beyond that, nsLTPs in many plants, such as those in tomato seeds, are regarded as allergens[17]. In conclusion, lipid transfer proteins are widely present in plants, but their functions have not yet been accurately elucidated.

Sweet potato(*Ipomoea batatas* L. (Lam.)), of the *Ipomoea* genus of the Convolvulaceae family, is an important agricultural crop in tropical developing countries. It ranks among the top seven most important food crops due to its high yield, rich nutrient content, and wide adaptability to various climates and farming systems. It is not only used as human food and animal feed, but also extensively in industrial processes, such as bioethanol fermentation. However, few studies on sweet potato have been conducted due to its complex hexaploid genome ($2n = 6x = 90$) compared with that of other main crops or model organisms. Sweet potato has a relatively large genome size of about 2200 to 3000 Mbp. So far, several transcriptomic databases of different sweet potato cultivars have been built[18-20], including that of 'Xushu 18' in our laboratory using combined *de novo* transcriptome assembly[21, 22]. Many sweet potato genes have been researched, including betaine aldehyde dehydrogenase[23], late embryogenesis abundant 14[24], starch branching enzymes[25] and transposase genes[26].

In this study, we cloned and characterized the nucleotide sequences of two *nsLTPs* (*IbLTP1* and *IbLTP2*) from *I. batatas* (L.) Lam., cv. Xushu 18. In addition, the expression patterns and expression differences of *IbLTP1* and *IbLTP2* in different tissues and under stress were determined by real-time RT-PCR. Results from this study will provide further understanding of the role of nsLTPs in plant stress tolerance.

1　Material & Methods

1.1　Plant material and treatment

I. batatas (L.) Lam., cv. Xushu 18, which has a long cultivation history in southern China, was used in this study. Stem cuts were planted in May, 2014 in an experimental field of the College of Life Sciences in Sichuan University, Chengdu, China. Two-month-old plants were well irrigated at their roots with 2 L of 200 mmol/L NaCl solution once every 3 d for 20 d. Control samples were grown under normal conditions.

We collected the leaves, stems, and roots from at least three plants after abiotic stress at 0, 3, 10 and 20 d as well as from the control. The harvested leaves, stems, and roots were cleaned with distilled water and frozen immediately in liquid nitrogen, then stored at −80℃ for further RNA or DNA preparation.

1.2　Total RNA and genomic DNA extraction and purification

Total RNA was extracted from different tissues using TRNzol Reagent (TIANGEN, China). RNA concentration was assessed with the OD_{260}/OD_{280} ratio using spectrophotometry, and the integrity of total RNA samples was identified using 1% agarose gel electrophoresis. cDNA was synthesized using a PrimeScript RT reagent kit with gDNA Eraser (TaKaRa, China) according to the manufacturer's instructions.

The CTAB method was used to extract and purify the genomic DNA of sweet potato. First, the plant tissues were ground into powder in liquid nitrogen using a deeply frozen mortar and pestle. The powder was then resuspended in the CTAB extraction buffer, which was preheated in 65℃, and incubated at 65℃ for 60 min. Chloroform/isoamyl alcohol (24 : 1) was added to remove the proteins. The mixture was then gently shaken and centrifuged at 4000 r/min for 20 min at room temperature. The mixture formed three layers from the top to bottom. The top aqueous phase was transferred into a new centrifuge tube. Isopropanol (2/3 of the top aqueous phase volume) was added and maintained for 10 min to precipitate DNA. The mixture was then centrifuged at 4000 r/min for 20 min at room temperature and the DNA pellet was resuspended using precooled 70% alcohol for washing. The mixture was centrifuged at 3000 r/min for 10 min at room temperature to harvest the DNA pellet, which was

then dried at 37℃ for 15 min. The DNA pellet was then dissolved using RNase-H_2O (20 μg RNase/mL) and stored at −20℃ for future use.

1.3 Isolation of *IbLTPs* and construction of the recombinant plasmid

The cDNA of *IbLTP1* and *IbLTP2* were amplified from the cDNA of sweet potato using PCR with primers IbLTP1F, IbLTP1R and IbLTP2F, IbLTP2R (Table 1), respectively. The primers for amplification were designed according to the sequences using Primer Premier 5.0 (Premier Biosoft International, CA, USA). The sequences were amplified using rTaq (TaKaRa, China) and the PCR products were separated in 2% agarose gel and recovered using a gel extraction kit (Omega Bio-Tek, USA) according to the manufacturer's instructions. The purified sequences were inserted into the plasmid vector pMD19-T (Tiangen, China) and then transformed in competent cells of *Escherichia coli* strain JM109. The positive monoclonal colonies were then selected and sequenced (Genewiz, China).

Primers PE1F, PE2F (with an *Eco*R Ⅰ site) and PE1R, PE2R (with a *Hin*d Ⅲ site) were used to amplify the CDS of *IbLTP1* and *IbLTP2*, respectively (Table 1). The PCR reactions were performed using DNA polymerase KOD-Plus-Neo (Toyobo, Japan) under the following conditions: 94℃ for 3 min, 30 cycles at 98℃ for 10 s, 50℃ for 30 s, and 68℃ for 30 s, followed by 68℃ for 10 min. The PCR products were purified using a Cycle-Pure kit (Omega Bio-Tek, USA) according to the manufacturer's instructions, and digested by enzymes *Eco*R Ⅰ and *Hin*d Ⅲ and then subcloned into the pET32a (+) vector, which was also digested by *Eco*R Ⅰ and *Hin*d Ⅲ. The recombinant plasmids pET32a-IbLTP1 and pET32a-IbLTP2 were obtained and transferred into *E. coli* strain BL21 (DE3). The positive recombinants were selected and identified by double enzyme digestion.

Table 1 Primers used in this study

Primer	Sequence (5′−3′)
IbLTP1F	TCCTGTCTTTCAATTCCCAGATC
IbLTP1R	GCGACACAGTACAGACCAG
IbLTP2F	GCTAAGCTAGCTTGGCAATCC
IbLTP2R	GTCCCATATATGATACCAACTCTC
PE1F	CCGGAATTCATGGCAAATCTTAGTTTGG
PE1R	CCCAAGCTTAGTGAACCTTGGAGCAG
PE2F	CCGGAATTCATGAGGAGCATAGCAATCTG
PR2R	CCCAAGCTTAGCAACGAGGAAAGGG
QRT-LTP1F	TAGTTTGGTTATTGCGGTGTTG
QRT-LTP1R	TTGTCCCTTGCCTGTGATGT
QRT-LTP2F	ATGAGGAGCATAGCAATCTG
QRT-LTP2R	GCAACCTTCTTGGCATTAG
β-actinF	CTGGTGTTATGGTTGGGATGGGAC
β-actinR	GAAGGACAGGGTGCTCCTCAGG

1.4 Sequence analysis of *IbLTPs*

Sequences were Blasted in NCBI (http://www.ncbi.nlm.nih.gov/) to analyze the conserved domains and active sites. ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) was used to find the open reading frames (ORF) of *IbLTP1* and *IbLTP2*. The physical and chemical parameters of IbLTP1 and IbLTP2 were computed using the ProtParam tool (http://web.expasy.org/protparam/). The presence and location of signal peptide cleavage sites in IbLTP1 and IbLTP2 were predicted using SignalP 4.1 (http://www.cbs.dtu.dk/services/SignalP/). Multiple sequence alignments and homology analysis were done using DNAMAN software. MEGA 5.10 software was used to construct the phylogenetic trees of IbLTP1 and IbLTP2 using maximum likelihood. The amino acid sequences of other plants, using to construct the phylogenetic trees, were downloaded from GenBank (Table 2).

1.5 Expression analysis of *IbLTPs* in different tissues and under NaCl stress

Real-time RT-PCR was used to detect gene expressions in different plant tissues and in response to NaCl stress. The β-*actin* gene was chosen as a reference to normalize the total RNA amount in each reaction. The ΔΔCt method was used to calculate relative gene expression. cDNA was prepared from young leaves (YL), mature leaves (ML), stems (ST), fibrous roots (FR),

Table 2　Species and accession numbers of LTPs sequences used for phylogenetic analysis

Species	Accession number	Definition
Ricinus communis L.	GI：255572170	Nonspecific lipid-transfer protein, putative
Vitis vinifera L.	GI：731370169	Predicted：non-specific lipid-transfer protein 2-like
Prunus mume (Siebold) Siebold & Zucc.	GI：645276420	Predicted：non-specific lipid-transfer protein 2-like
Citrus sinensis L. Osbeck	GI：568828853	Predicted：non-specific lipid-transfer protein 2
Nicotiana tomentosiformis Goodsp.	GI：697119589	Predicted：non-specific lipid-transfer protein 2-like
Solanum lycopersicum L.	GI：723697796	Predicted：non-specific lipid-transfer protein 2-like
Sesamum indicum L.	GI：747042034	Predicted：non-specific lipid-transfer protein 2-like
Cucumis melo L.	GI：659066780	Predicted：non-specific lipid-transfer protein 2
Cicer arietinum L.	GI：502163612	Predicted：non-specific lipid-transfer protein 2
Medicago truncatula Gaertn.	GI：357500235	Chitinase / Hevein / PR-4 / Wheatwin2
Glycine soja Sieb. et Zucc.	GI：734428064	Putative non-specific lipid-transfer protein AKCS9
Beta vulgaris subsp. *vulgaris* L.	GI：731344615	Predicted：non-specific lipid-transfer protein-like
Solanum chacoense Bitter.	GI：91107174	lipid-transfer protein precursor
Lactuca sativa L.	GI：118490068	lipid transfer protein isoform 1.1 precursor
Davidia involucrate Baill.	GI：16903206	lipid transfer protein precursor
Gossypium herbaceum subsp. *africanum* (Watt) Vollesen	GI：403391437	lipid transfer protein precursor
G. hirsutum L.	GI：7012724	lipid transfer protein precursor
Vitis pseudoreticulata W. T. Wang	GI：390985898	nonspecific lipid transfer protein
Gymnadenia conopsea L. R. Br.	GI：109255207	phosophlipid protein
Fragaria x ananassa (Weston) Duchesne ex Rozier	GI：67937769	non-specific lipid transfer protein precursor

initial tuberous roots (IR) under normal conditions, and from the leaves, stems, and roots under NaCl stress at different times. All real-time PCR runs were performed in triplicate (technical replicates) and each reaction mixture was prepared using a SYBR Premix Ex *Taq* kit (TaKaRa, China). The reaction mixture (20 μL) contained 10 μL of 2 × SYBR Premix Ex *Taq*, 6.4 μL of PCR-grade water, 0.8 μL of each forward and reverse primer, and 2.0 μL of appropriately diluted template cDNA. The procedure was: 95℃ for 3 min followed by 40 cycles at 95℃ for 30 s, 58℃ for 30 s, 72℃ for 30 s and 82℃ for 1 s for plate reading. After amplification, a melting peak analysis with a temperature gradient of 0.1℃ per second from 60 to 95℃ was performed to confirm that only the specific products were amplified. These procedures were optimized for 96-well format using a Bio-Rad IQ detection system with fluorescein as an internal passive reference dye for normalization of well-to-well optical variation.

1.6　Response of recombinant BL21 (DE3) to NaCl stress

The recombinant BL21 was grown in LB medium containing 600 mmol/L NaCl and 100 μg/mL of ampicillin at 37℃ with shaking at 140 r/min. When the OD_{600} reached 0.5, 0.2 mmol/L isopropyl-thiogalactoside (IPTG) was added to the medium to induce protein expression. The control BL21 strains were grown in normal LB medium containing 100 μg/mL of ampicillin only. The OD_{600} values of the cultures were recorded every 3 h and 2 h, respectively. Each experiment was repeated in triplicate.

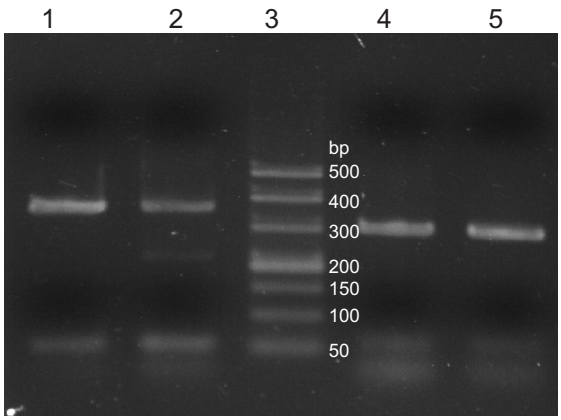
2　Results

2.1　Cloning and sequence analysis of *IbLTP1* and *IbLTP2*

Through scanning the transcriptome database established in our laboratory, we obtained many *nsLTP* contigs. Two contigs (Contig_11802, Contig_14035) were researched in this study. Sequence analysis showed that they encoded a protein homologous to other lipid transfer proteins in the NCBI database, and so were named as *IbLTP1* and *IbLTP2*, respectively. Their cDNA with 5′ and 3′ UTR were amplified from sweet potato cDNA and inserted into the plasmid

vector pMD19-T. The recombinant plasmids were transformed in competent cells of *E. coli* strain JM109 and the positive monoclonal colonies were selected and sequenced.

The sequencing results showed that *IbLTP1* contained a 345 pb ORF and *IbLTP2* contained a 285 bp ORF. In addition, *IbLTP1* and *IbLTP2* had four and three homologous isoforms, respectively. The PCR products of *IbLTP1* and *IbLTP2* were also sequenced to ensure that these isoforms were not due to experimental errors. If there were different bases among the homologous isoforms, the peak diagrams of PCR products showed double peaks at the same base location. The results demonstrated that there were 24 and 8 SNP sites in *IbLTP1* and *IbLTP2*, respectively. The genomic DNA of *IbLTP1* and *IbLTP2* were also amplified with the same primers from sweet potato genomic DNA. The sequence comparisons of genomic DNA and cDNA showed there were no introns in the ORFs of *IbLTP1* or *IbLTP2* (Fig. 1).



1, 2: cDNA and genomic DNA of *IbLTP1*; 3: DNA marker; 4, 5: cDNA and genomic DNA of *IbLTP2*.

Fig. 1 PCR amplification of cDNA and genomic DNA of *IbLTP1* and *IbLTP2*

2.2 Sequence and phylogenetic analyses of IbLTP1 and IbLTP2 proteins

IbLTP1 contains 114 amino acid residues, including three negatively charged residues (Asp + Glu) and 10 positively charged residues (Arg + Lys) with a molecular weight of 11.2 kD and pI of 9.07. IbLTP2 contains 94 amino acid residues, including five negatively charged residues (Asp + Glu) and 11 positively charged residues (Arg + Lys) with a molecular weight of 10 kD and pI of 8.97. Neither contained any Trp residues. Using SignalP 4.1, the putative protein of IbLTP1 was predicted to contain a typical signal peptide of 22 amino acid residues at the N-terminus, whereas IbLTP2 was predicted to contain a signal peptide of 25 amino acid residues. They contained eight strictly conserved cysteines, which formed four disulfide bridges. Conserved domain and active site analyses demonstrated that IbLTP1 had an nsLTP1 conserved domain and IbLTP2 had an nsLTP2 conserved domain. Therefore, IbLTP1 and IbLTP2 belonged to Type Ⅰ and Type Ⅱ, respectively. Multiple amino acid sequence alignments showed that IbLTP1 shared 61.40% amino acid identity with *Gymnadenia conopsea* LTP (GI: 109255207), whereas IbLTP2 shared 71.70% amino acid identity with *Nicotiana tomentosiformis* LTP (GI: 697119589). Based on multiple amino acid sequence alignments, we constructed a phylogenetic tree to show the relationship between IbLTPs and other plant LTPs (Fig. 2).

2.3 Expression pattern analysis of *IbLTP1* and *IbLTP2* in different tissues

The gene expression levels of *IbLTP1* and *IbLTP2* in different tissues are shown in Fig. 3. The expression levels of genes in FR were used as a calibrator (designated as 1) to determine relative expression levels. *IbLTP1* had the highest expression level in young leaves; *IbLTP2* displayed the highest expression level in stems, which was 2-fold higher than that in other tissues. *IbLTP1* exhibited the lowest expression level in roots, whereas *IbLTP2* showed the lowest expression level in mature leaves. There was no differential expression of *IbLTP2* between young leaves and fibrous roots.

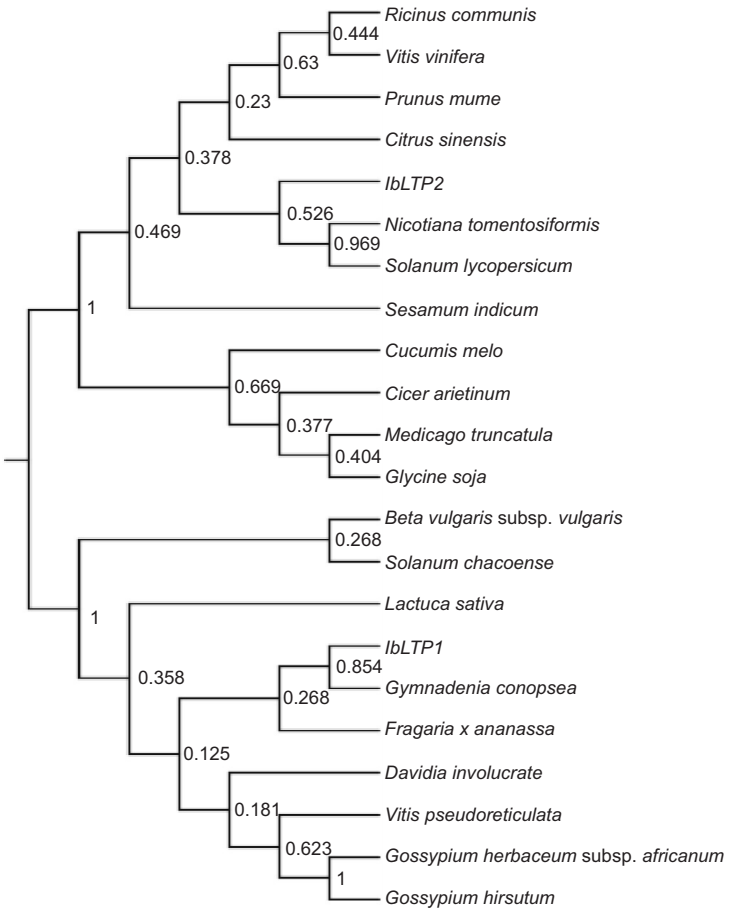
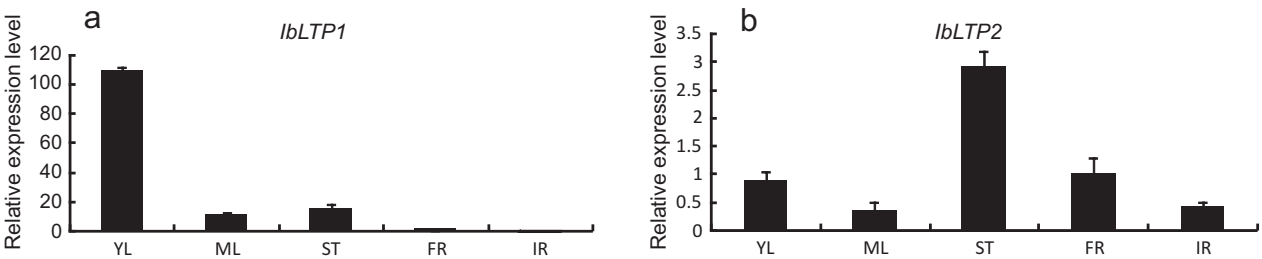


Fig. 2　Phylogenetic tree of IbLTPs and homologous LTPs from other plants



Expression levels of (a) *IbLTP1* and (b) *IbLTP2* in young leaves (YL), mature leaves (ML), stems (ST), fibrous roots (FR) and initial tuberous roots (IR). Expression level of genes in FR was used as a calibrator (designated as 1) to determine relative expression levels.

Fig. 3 qRT-PCR expression patterns of *IbLTPs* in different tissues

2.4　Expression of *IbLTP1* and *IbLTP2* under NaCl stress

To elucidate the possible role of *IbLTP1* and *IbLTP2* in sweet potato, their expression patterns in response to NaCl stress were analyzed using qRT-PCR. The expression level of genes on 0 d were used as a calibrator (designated as 1) to determine the relative expression levels. The results showed that there were no changes in the expression levels of *IbLTP1* and *IbLTP2* in roots under NaCl stress after 3 d, 10 d and 20 d compared with that on 0 d (Fig. 4: a). In stems, the expression of *IbLTP1* and *IbLTP2* was clearly up-regulated by NaCl stress, showing a 3-fold increase after 3 d of treatment. These responses continued after 10 d and 20 d of treatment (Fig. 4:

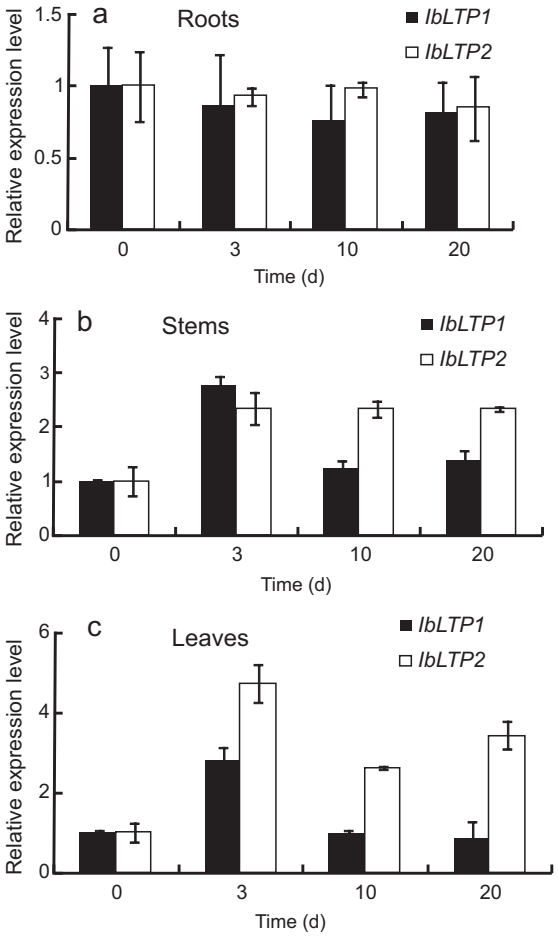


Fig. 4 Time-course expression analysis of *IbLTPs* responding to NaCl stress in (a) roots, (b) stems and (c) leaves

b). In leaves, the expressions of *IbLTP1* and *IbLTP2* were also upregulated respectively, showing a 3-fold and 5-fold increase after 3 d treatment, with the induction of *IbLTP2* persisting after 10 d and 20 d of treatment (Fig. 4: c).

2.5 Response of recombinant *E. coli* to NaCl stress

To verify the effect of IbLTP1 and IbLTP2 proteins on the growth of host cells under NaCl stress, the recombinant plasmids pET32a-IbLTP1 and pET32a-IbLTP2 were constructed and expressed in *E. coli*. Under normal conditions the growth curves were roughly the same between the recombinant and control strains (Fig. 5: a). Under NaCl stress, however, all bacterial strains displayed slower growth than that under normal conditions and the maximal OD_{600} values of strains harboring pET32a-IbLTP1 and pET32a-IbLTP2 were higher than that of the pET32a-transformed strain (Fig. 5: b), indicating that both expressed *IbLTPs* conferred the host cells salt-tolerance.

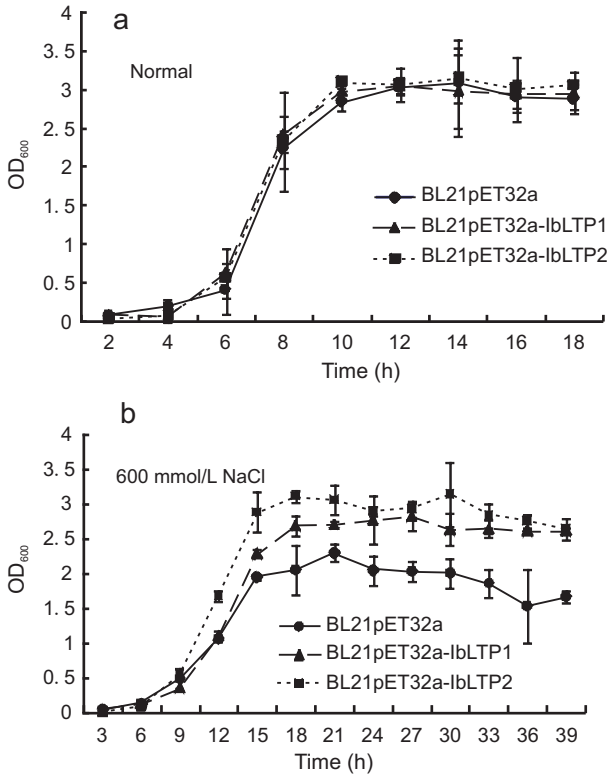


Fig. 5 Growth curves of *E. coli* BL21 (DE3) strains harboring recombinant plasmid pET32a-IbLTP1, pET32a-IbLTP2 or pET32a in (a) LB medium and (b) LB medium with 600 mmol/L NaCl

3 Discussion

Sweet potato is constantly exposed to abiotic stress, with drought and salt being particularly serious. Thus, plants usually upregulate or downregulate the expression of numerous genes in response to these stresses, including late embryogenesis abundant 14[24], betaine aldehyde dehydrogenase gene (*IbBADH*)[23], and multiple stress responsible gene Ⅰ (*MuSI*)[27]. In this study, we analyzed the expression patterns of *IbLTP1* and *IbLTP2* under NaCl stress.

According to the conserved domain and active site analyses, the deduced protein sequence

of IbLTP1 contained the nsLTP1 conserved domain and that of IbLTP2 contained the nsLTP2 domain. Similar to other nsLTPs, they have basic isoelectric points, lower molecular weights, and contain eight strictly conserved cysteine residues and signal peptides, suggesting that they belong to the LTP family.

Usually, *nsLTP* genes have different expression patterns in different plant tissues. The highest expression level of *IbLTP1* was found in young leaves, in accordance with previous results in which the expression level of *nsLTP* in young leaves of broccoli was higher than that in mature leaves[28]. A similar expression pattern was also found in *Arabidopsis*[29]. *IbLTP1* may play a role in the formation and deposition of cuticular components. The relatively high expression level of *IbLTP1* in leaves suggests that it may play a more important role in the physiological processes in leaves than that in other tissues. However, the expression of *IbLTP2* was higher in stems than that in other tissues, indicating a more important function in stems.

The expression level of *LTP* genes under salt stress has been studied in many plants[11, 12]. In this study, we found that *IbLTP1* and *IbLTP2* were regulated by NaCl stress. Real-time RT-PCR results showed that *IbLTP1* and *IbLTP2* were upregulated more than 2-fold in stems and leaves after 3 d treatment, indicating that both are likely involved in salt stress response. The duration of the upregulation of *IbLTP2* was longer than that of *IbLTP1* in leaves and stems. These results suggested that the physiological importance of *IbLTP2* may be higher than that of *IbLTP1* in NaCl stress response. Prokaryotic expression systems are an effective way to express exogenous protein. We elucidated the contribution of *IbLTPs* to NaCl stress by treating recombinant *E. coli* harboring *IbLTPs* with NaCl stress. The expression of *IbLTPs* enhanced the salt tolerance of the host cell.

In this study, two *nsLTP* genes were cloned and named *IbLTP1* and *IbLTP2*. Their expression patterns in different tissues under normal growth conditions and NaCl stress were examined. Results indicated that *IbLTP1* and *IbLTP2* had higher relative expression levels in young leaves and stems, respectively, and were highly induced under NaCl stress. However, the role of *IbLTPs* in plant physiology remains unclear. In future research, the function of *IbLTPs* should be accurately elucidated.

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