Plant Mol Biol Rep (2013) 31:991–1001
DOI 10.1007/s11105-013-0571-6

ORIGINAL PAPER

Isolation and Characterization of a Lipid Transfer Protein Gene (*BplLTP1*) from *Betula platyphylla*

Minxiao Guan · Ruihai Chai · Xue Kong · Xuemei Liu

Published online: 24 February 2013

Abstract Although nonspecific lipid transfer proteins (nsLTPs) are widely present in plants, their functions are not fully understood. Here, we isolated and characterized a putative nsLTP gene, *BplLTP1*, from *Betula platyphylla*. The full-length cDNA of *BplLTP1* is 638 bp long, including a 363-bp open reading frame (GenBank accession no. JQ409562). The putative protein BplLTP1 contains an N-terminal signal sequence and possesses the characteristic features of nsLTPs. An amino acid sequence alignment revealed that BplLTP1 shares a high level of similarity with other known nsLTPs. A 3D model of BplLTP1 was also constructed based on the homology model. Quantitative real time-PCR analysis showed that there were no obvious differences in the expression levels of *BplLTP1* among different tissues. *BplLTP1* displayed distinctly higher expression levels in young tissues than in older tissues. Moreover, *BplLTP1* was upregulated at the mononuclear microspore developmental stages in male inflorescences. Expression analysis was performed using 3-month-old cultured seedlings, and the results revealed that the expression of *BplLTP1* was upregulated by exogenous abscisic acid and salicylic acid, downregulated by exogenous methyl jasmonate, and not significantly altered by exogenous gibberellin A. In addition, a prokaryotic expression system was constructed with pET32a-BplLTP1 and *Escherichia coli* strain BL21 and subjected to abiotic stress resistance analysis. The results indicated that the expression of *BplLTP1* improved the resistance of the recombinant strain to salt (NaCl) and drought (polyethylene glycol) stress, but not to alkali ($NaHCO_3$) stress.

Keywords *Betula platyphylla* · Nonspecific lipid transfer protein (nsLTP) · Prokaryotic expression · Abiotic stresses · Exogenous hormone

M. Guan · R. Chai · X. Kong · X. Liu (✉)
Northeast Forestry University, Harbin 150040,
People's Republic of China
e-mail: liuxuemei@nefu.edu.cn

Introduction

Plants are frequently subjected to a plethora of abiotic stress conditions, such as drought, flood, salt, alkali, low temperature, heat, and heavy metal toxicity. Plants are also subjected to various biotic challenges from pathogens and herbivores. All of these abiotic and biotic stress factors have adverse effects on plant growth and productivity. In response to these stress factors, various genes are regulated to mitigate the effects of stress and to enhance plant tolerance (Mahajan and Tuteja 2005; Knight and Knight 2001).

Lipid transfer proteins (LTPs) are widely present in animals, plants, and microorganisms (Yamada 1992) and account for approximately 4 % of the total soluble protein content in higher plants (Kader 1996). Plant nonspecific lipid transfer proteins (nsLTPs), which were first identified in 1984, are characterized by their capacity to facilitate the transfer of phospholipids between liposomes in vitro (Kader et al. 1984). To date, plant nsLTPs have been identified in 50 different species, and there are more than 100 potential plant nsLTPs (José-Estanyol et al. 2004). Plant nsLTPs are cysteine-rich peptides with low molecular masses encoded by a multigene family. Plant nsLTPs are mainly grouped into two subfamilies based on their primary structures (Carvalho and Gomes 2007; Yeats and Rose 2008; Arondel et al. 2000). nsLTP1 members have molecular masses of approximately 9 kDa and contain 90–95 amino acid residues, while nsLTP2 members have molecular masses of approximately 7 kDa and possess, on average, 70 amino acids. Both nsLTP1 and nsLTP2 family members are abundant, basic proteins with isoelectric points (p*I*) between 8.5 and 12, with eight strictly conserved cysteine residues (Kader 1997; Carvalho and Gomes 2007). Both nsLTP1 and nsLTP2 family members contain a signal peptide at the amino terminal region, generally comprising 21–27 amino acids for the nsLTP1 family and 27–35 amino acids for the nsLTP2 family, allowing mature LTP peptides to be exported to the apoplast accurately

(Carvalho and Gomes 2007). The 3D structure of the plant nsLTP family comprises a globular molecule stabilized by four disulfide bridges (Shin et al. 1995; Samuel et al. 2002). The most remarkable structural feature of the LTP family is the presence of a hydrophobic cavity that has the capacity to bind fatty acids, acyl-coenzyme A (acyl-CoA), and phospholipids (Carvalho and Gomes 2007).

Several plant nsLTP1 members have been localized to the cell wall (Thoma et al. 1993; Pyee et al. 1994; De O. Carvalho et al. 2004) and have been extracted from plant surfaces (Pyee et al. 1994). Tissue-specific expression experiments examining nsLTPs have demonstrated that nsLTP genes are expressed at different levels in various tissues during diverse developmental stages (Pyee et al. 1994; Thoma et al. 1994). Plant nsLTP genes are responsive to abiotic stresses such as drought, cold and salt stress, and biotic stresses such as bacterial and fungal pathogens (Jang et al. 2004; Jung et al. 2003; Wu et al. 2004; Wang et al. 2009; Sarowar et al. 2008). In addition, several signaling molecules such as abscisic acid (ABA), salicylic acid (SA), ethylene, and methyl jasmonate (MJ) are also able to regulate the expression of nsLTP genes (Yubero-Serrano et al. 2003; Wu et al. 2004; Jung et al. 2003; Jung et al. 2006; Wang et al. 2009). Furthermore, a variety of potential biological functions for nsLTPs have also been proposed, such as surface cutin biosynthesis (Kader 1996; DeBono et al. 2009; Lee et al. 2009), liquid secretion (Choi et al. 2012), embryogenesis (Kader 1996), anther development (Kader 1996; Ariizumi et al. 2002; Zhang et al. 2010; Chae et al. 2009; Chen et al. 2011), pollen tube tip growth (Chae et al. 2009), seed maturation (Thoma et al. 1994), and plant signaling (Gao et al. 2009; Sarowar et al. 2008; Blein et al. 2002), although the exact biological role of nsLTPs in vivo remains unclear.

Asian white birch (*Betula platyphylla*) is an important economic forest tree species in northern China. In this study, we isolated and characterized a putative nsLTP gene, *BplLTP1*, from *B. platyphylla* based on the expressed sequence tag (EST) method, which was obtained from a cDNA-AFLP library created from the early and late developmental stages of male inflorescences (Xing and Liu 2011). We investigated the expression patterns of *BplLTP1* in various tissues and the diverse developmental stages of male inflorescences. The *BplLTP1* expression patterns under ABA, SA, MJ, and gibberellin A (GA_3) treatment were also examined. In addition, a prokaryotic expression system was constructed using pET32a-BplLTP1 and *Escherichia coli* strain BL21 and subjected to NaCl, PEG 6000 (polyethylene glycol), and $NaHCO_3$ stress resistance analyses. Taken together, the results of this study suggest that *BplLTP1* plays an important role in the defense responses of *B. platyphylla*.

Materials and Methods

Plant Material and Treatment

Plant materials, which were used to clone *BplLTP1* and analyze tissue-specific expression, were sampled from mature *B. platyphylla* in the birch forest yard of Northeast Forestry University, Heilongjiang, China. Male inflorescences were sampled every 2–3 days from July 15 to September 1, 2011. From July 15 to August 14, the male inflorescences were in the meiosis stages, and from August 23 to September 1, they were in the mononuclear microspore developmental stages (Liu and Yang 2006). Mature pollen and seeds were sampled on May 6 and August 15, respectively. Female inflorescences, male inflorescences, young leaves, older leaves, young petioles, older petioles, young stems, and older stems were sampled on July 15. The samples were quickly frozen in liquid nitrogen and stored at −80 °C before total RNA extraction.

Birch twigs with buds were cultivated in vitro in a rooting medium consisting of woody plant medium (Lloyd and McCown 1980) supplemented with 2 μM indole-3-butytric acid. The cultures were maintained under a 16-h light/8-h dark photoperiod. When the plants were 3 months old, they were sprayed with GA_3 (500 μM), SA (1 mM), ABA (100 μM), and MJ (100 μM). The plants were sampled 0 (control), 4, 8, 12, or 24 h after treatment. The leaves were harvested and frozen in liquid nitrogen.

Total RNA Extraction and Purification

Total RNA was extracted from different tissues using the modified CTAB method (Chang et al. 1993), and the potential contaminating genomic DNA was digested at 37 °C for 30 min using DNase I (Takara, Japan). The integrity of the total RNA samples was verified using 1 % agarose gel electrophoresis.

Isolation of BplLTP1

To obtain the full-length cDNA sequence of *BplLTP1*, 5′ rapid amplification of cDNA ends (RACE) and 3′ RACE reactions were performed with a 5′ and 3′ Full RACE Kit (Takara) according to the manufacturer's protocol. Briefly, for 5′ RACE, the 5′ cap structure was removed from purified RNA using tobacco acid pyrophosphatase, ligated to the 5′ RACE adaptor using T4 DNA ligase, and used as a template to synthesize first-strand cDNA using M-MLV reverse transcriptase (Takara). Two specific primers (5′GSP1 and 5′ GSP2; Table 1) were designed. Primer 5′GSP1, along with the 5′ RACE Outer Primer, was used for the primary PCR. The primary PCR products were then diluted 100-fold with deionized water to be used as the template for the nested

PCR. Primer 5′GSP2 and the 5′ RACE Inner Primer were used for the nested PCR. The PCR reactions were performed as follows: 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 61 °C (for the primary PCR) or 65 °C (for the nested PCR) for 30 s and 72 °C for 1 min, followed by 72 °C for 10 min. The PCR products were purified and subcloned into the pMD18-T vector (Takara) and sequenced by the Beijing Genomic Institute (Beijing, China). For 3′ RACE, the purified RNA was used to synthesize first-strand cDNA using M-MLV reverse transcriptase (Takara) with the oligo-dT-3 site adaptor primer. The primary PCR was performed with 3′GSP1 and the 3′ RACE Outer Primer (Table 1). The PCR products were then diluted and used as templates for the nested PCR with 3'GSP2 and 3'RACE Inner Primer (Table 1). The PCR reactions were performed as follows: 94 °C for 5 min, 30 cycles of 94 °C for 30 s, 63 °C (for the primary PCR) or 66 °C (for the nested PCR) for 30 s and 72 °C for 1 min, followed by 72 °C for 10 min. The 3′ RACE PCR products were purified, subcloned into the pMD18-T vector (Takara), and sequenced.

Sequence Analysis of BplLTP1

The open reading frame (ORF) of the *BplLTP1* cDNA sequence was analyzed using the ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/). The p*I* (isoelectric point) and M_w (molecular weight) predictions were performed using the Compute p*I*/Mw tool (http://web.expasy.org/compute_pi/; Bjellqvist et al. 1993). Conserved domains were identified using the NCBI Conserved Domain Database (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi/; Marchler-Bauer et al. 2011). The signal peptide was predicted using online SignalP, version 4.0 (http://www.cbs.dtu.dk/services/SignalP/; Petersen et al. 2011). Multiple sequence alignments were carried out with ClustalX, version 1.83 (Thompson et al. 1997). The phylogenetic tree was constructed based on MEGA 5 (Tamura et al. 2011). Homology modeling of the 3D structure of BplLTP1 was generated using the SWISS-MODEL server (http://swissmodel.expasy.org/; Arnold et al. 2006).

Quantitative and Semiquantitative RT-PCR

The cDNA sample was synthesized from 1 μg of total RNA using M-MLV reverse transcriptase (Takara). After first-strand cDNA synthesis, the cDNA sample was used as a template for quantitative RT-PCR (qRT-PCR) or semiquantitative RT-PCR analysis. qRT-PCR was performed using an MJ OpticonTM2 System (Bio-Rad, Hercules, CA) and QuantiTect SYBR-green PCR Master Mix (Toyobo, Japan). The qRT-PCR reactions were performed as follows: 95 °C for 5 min followed by 40 cycles at 95 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s, and 79 °C for 1 s. Semiquantitative RT-PCR was performed at 94 °C for 5 min followed by 26 cycles at 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. The *Actin* gene (GenBank accession no. EU588981) was used as the standard control in quantitative and semiquantitative RT-PCR (Qi et al. 2010; Li et al. 2010). The primer sequences are shown in Table 1. These experiments were performed in triplicate.

Response of Recombinant BL21 (DE3) to Abiotic Stresses

Primers PF (with a *Bam*HI site) and PR (with a *Sac*I site; Table 1) were used to amplify the ORF of *BplLTP1* without the N-terminal signal peptide sequence. The PCR reactions

Table 1 Primers used in this study

Names	Sequence (5′–3′)	Description
5′GSP1	TAACATTACACTTGCCAGGGAGG	5′ RACE specific primer, outer
5′GSP2	GGGGTGGTCTTGGCAGCATTGTTG	5′ RACE specific primer, nested
5′ Outer Primer	CATGGCTACATGCTGACAGCCTA	5′ RACE universal primer, outer
5′ Inner Primer	CGCGGATCCACAGCCTACTGATGATCAGTCGATG	5′ RACE universal primer, nested
3′GSP1	GTCAACAATGCTGCCAAGACCACCC	3′ RACE specific primer, outer
3′GSP2	GGCCGCTTCTGCCTTATCCGGTGTC	3′ RACE specific primer, nested
3′ Outer Primer	AGGATCCGCGGACTAGTG$(T)_{11}$	3′ RACE universal primer, outer
3′ Inner Primer	AGGATCCGCGGACTAGTG	3′ RACE universal primer, nested
QF	GCTAGCTCCATCGTCCT	Quantitative and semiquantitative RT-PCR, forward
QR	GTTTGAACCTGCCCACAT	Quantitative and semiquantitative RT-PCR, reverse
ActinF	CATCTCTGATCGGAATGGAAG	Standard control primer, forward
ActinR	AGATCCTTTCTGATATCCACG	Standard control primer, reverse
PF	GGATCCATGGCCGTGTCATCGTCC	Recombinant plasmid primer, forward
PR	GAGCTCCTCATTTAATGGTTTTG	Recombinant plasmid primer, reverse

were performed under the following conditions: 95 °C for 5 min, 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 1 min, followed by 72 °C for 10 min. The PCR products were subcloned into the pMD18-T vector (Takara) and transferred to *E. coli* DH5α competent cells. After sequencing identification, the recombinant plasmids were digested with the enzymes *Bam*HI and *Sac*I and subcloned into the bacterial expression vector pET-32a(+) (Novagen) that had been digested with the same enzymes, which resulted in a recombinant plasmid named pET32a-BplLTP1. Both pET-32a (+; blank vector) and pET32a-BplLTP1 were transformed into *E. coli* strain BL21 (DE3). The bacterial cultures were grown in Luria–Bertani (LB) medium (100 $\mu g ml^{-1}$ ampicillin) at 37 °C until the culture optical density values at 600 nm (OD_{600}) reached 0.6, as determined with an ultraviolet spectrophotometer (Shimadzu, China). One millimolar isopropyl-thiogalactoside (IPTG) was used to induce protein expression. After induction for 4 h, both pET-32a and pET32a-BplLTP1 bacterial cultures were added to fresh liquid LB medium (100 $\mu g ml^{-1}$ ampicillin) supplemented with 0.4 M NaCl, 0.2 M $NaHCO_3$, and 20 % PEG 6000 at a dilution of 1:100 and incubated at 37 °C with shaking (250 rpm). The OD_{600} values of the cultures were recorded every 1.5 h. Untreated bacterial strains were used as controls. Each experiment was performed with three parallel replicates.

Statistical Analysis

Statistical analyses were carried out using the IBM SPSS-19 statistical software package (IBM, USA). Results are represented as the mean ± SE. The data were analyzed statistically using ANOVA, and the differences between different samples were analyzed using the LSD test at a probability level of 0.05.

Results

Clone and Sequence Analysis of BplLTP1

To obtain the full-length cDNA of *BplLTP1*, 5′ and 3′ RACE-PCRs were performed using nested gene-specific primers. The full-length cDNA of *BplLTP1* (GenBank accession no. JQ409562) was 638 bp in length and contained a 363-bp ORF with a 67-bp 5′ UTR (untranslated region) and a 208-bp 3′ downstream UTR, based on the sequences of two overlapping frames obtained by the 5′ and 3′ RACE-PCR procedures. The *BplLTP1* ORF is predicted to encode a polypeptide of 120 amino acid residues. The putative polypeptide contains a typical signal peptide of 26 amino acid residues at the N terminus (Fig. 1), as determined using SignalP, version 4.0. After removing the signal peptide sequence, the mature peptide has a predicted molecular mass of 9.54 kDa, with a theoretical p*I* of 9.18.

Multiple amino acid sequence alignments showed that BplLTP1 shares identities with several other known nsLTP1s. BplLTP1 shares 64.2 % amino acid identity with *Gossypium hirsutum* LTP (GenBank accession no. AAF35185), and it shares 40.9 % amino acid identity with *Oryza sativa* LTP (GenBank accession no. ACA50499; Table 2). In addition, we also compared the homology between BplLTP1 and the LTP (GenBank accession no. EIL54273) of *E. coli*, which contains 392 amino acid residues and is not an nsLTP. *E. coli* LTP shares only 18.3 % homology with BplLTP1 (Table 2).

Further analysis showed that BplLTP1 contains the conserved in vitro lipid-binding motifs DRQ and CGV (Botton et al. 2002), and the eight strictly conserved cysteine residues form four disulfide bridges (Kader 1997; Fig. 1). To investigate the relationship between BplLTP1 and other plant nsLTP1 family proteins, a phylogenetic tree was constructed based on multiple amino acid sequence alignments. The phylogenetic tree shows that BplLTP1 is closely related to nsLTP1 from *Castanea sativa* (Fig. 2).

Homology modeling of the 3D structure of BplLTP1 was performed based on the template Pru p 3 (PDB code 2algB, resolution 2.30 Å) from *Prunus persica* (Pasquato et al. 2006). BplLTP1 shows 67.7 % amino acid sequence identity with *P. persica* Pru p 3 at an *E* value cutoff of 2.2e−21 (Arnold et al. 2006). The hypothetical 3D structural model of BplLTP1 shows a protein that contains four α-helices, with a secondary structure compacted by four disulfide bridges and a C-terminal tail. The four bridges are formed by Cys4 and Cys53, Cys14 and Cys30, Cys31 and Cys76, and Cys51 and Cys90 (Fig. 3).

Expression Patterns of BplLTP1 in Various *B. platyphylla* Tissues

The results of qRT-PCR analysis showed that the expression level of *BplLTP1* was significantly different in different tissues. *BplLTP1* expression was high in the leaves and male inflorescences, with the highest expression level in young leaves, while the transcripts were almost undetectable in the older stems and mature pollen. Interestingly, the results also showed that the expression level of *BplLTP1* was significantly higher in young tissues than in older tissues (Fig. 4a). We also examined the expression patterns of *BplLTP1* at different stages of male inflorescence development. The results (Fig. 4b) showed that *BplLTP1* expression was reduced by 57 % ($P<0.05$) at the meiosis stages, and the expression of this gene was significantly ($P<0.05$) increased (threefold) at the mononuclear microspore developmental stages (Liu and Yang 2006).

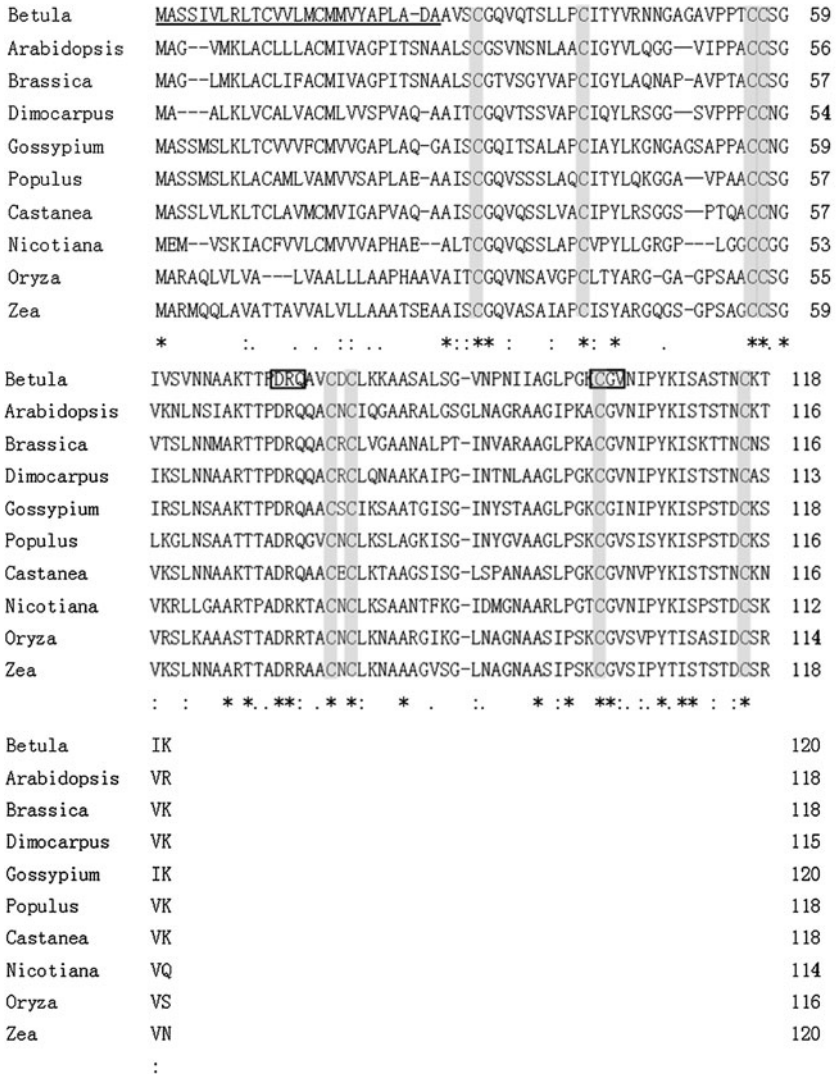

Fig. 1 Alignment of the deduced BplLTP1 sequence with other known plant nsLTP sequences. The amino acid sequences used in the alignment were from *Arabidopsis thaliana* (AAF76927), *Brassica oleracea* (AAA73948), *Dimocarpus longan* (AEC04836), *Gossypium hirsutum* (AAF35185), *Populus trichocarpa* (XP_002305877), *Castanea sativa* (ADK60918), *Nicotiana tabacum* (BAK19150), *Oryza sativa* (ACA50499) and *Zea mays* (ABA33846). The probable signal peptide sequence is *underlined*. The eight strictly conserved cysteine residues are shaded in *gray*. The two highly conserved regions (DRQ and CGV) in BplLTP1 are *boxed*. *Asterisks* indicate identical amino acids, *colon* indicates strongly similar, and a *period* indicates weakly similar amino acids

Response to Different Exogenous Plant Hormones

To elucidate the possible role of BplLTP1 in *B. platyphylla*, the expression patterns of *BplLTP1* in response to exogenous ABA, SA, MJ, and GA_3 were analyzed using semiquantitative RT-PCR. The results revealed that *BplLTP1* transcript levels increased significantly after 4 h of exogenous ABA treatment, and this elevated level was maintained (Fig. 5a). Under

Table 2 Comparison of the amino acid sequence percent identity of BplLTP1 with other plant nsLTPs and *E. coli* LTP

	1	2	3	4	5	6	7	8	9	10	11
1. BplLTP1 (*B. platyphylla*)		53.4	46.5	40.9	45.8	64.2	51.7	58.5	60.9	61.9	18.3
2. AAF76927 (*A. thaliana*)			48.7	46.9	49.6	52.6	69.2	54.3	58.3	55.2	17.8
3. BAK19150 (*N. tabacum*)				53.2	54.0	55.3	46.9	56.1	54.9	57.0	17.5
4. ACA50499 (*O. sativa*)					72.4	47.0	41.2	50.4	50.9	51.3	16.4
5. ABA33846 (*Z. mays*)						50.0	44.8	54.7	55.3	55.6	15.8
6. AAF35185 (*G. hirsutum*)							50.9	66.1	66.1	65.3	19.2
7. AAA73948 (*B. oleracea*)								52.6	62.6	50.9	17.8
8. XP_002305877 (*P. trichocarpa*)									60.9	61.0	20.3
9. AEC04836 (*D. longan*)										67.0	20.0
10. ADK60918 (*C. sativa*)											16.9
11. EIL54273 (*E. coli*)											

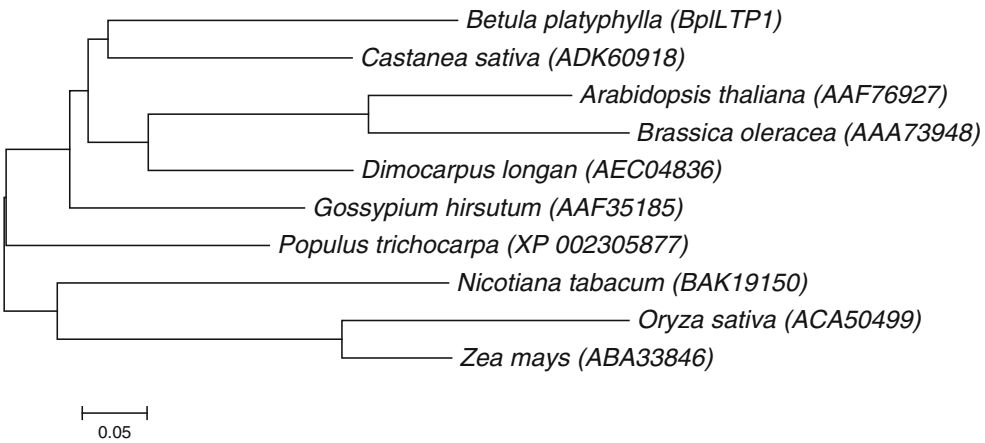

Fig. 2 Phylogenetic tree of BplLTP1 and homologous nsLTPs from other plants. The phylogenetic tree was generated with MEGA 5 software using the neighbor-joining method (Tamura et al. 2011). *Scale bar* represents evolutionary distance

exogenous SA treatment, *BplLTP1* transcript levels gradually increased (Fig. 5b). In contrast, the levels of the *BplLTP1* transcripts decreased significantly after 4 h of exogenous MJ treatment, followed by an increase after 16 h of treatment (Fig. 5c). Compared with ABA, SA, and MJ, exogenous GA_3 did not significantly alter the expression level of *BplLTP1* (Fig. 5d).

Response of Recombinant BL21 (DE3) to Abiotic Stresses

After 1 mM IPTG induction for 4 h, transformed *E. coli* BL21 (DE3) strain pET32a-BplLTP1 and the control strain pET32a containing an empty vector were cultured at 37 °C in LB medium. The OD_{600} values of the cultures were measured every 1.5 h. As shown in Fig. 6a, both bacterial strains presented typical "S" growth curves, which were substantially overlapping. The maximum OD_{600} values were both close to 2 during the stationary phase. These results suggest that the expression of *BplLTP1* rarely affects the normal growth of recombinant *E. coli* BL21 (DE3).

To determine the function of the fusion protein under salt (NaCl), alkali ($NaHCO_3$), and drought (PEG 6000) stress, the growth curves of *E. coli* BL21 (DE3) cultures harboring pET32a-BplLTP1 or pET32a were examined. Under salt stress, *E. coli* strain pET32a-BplLTP1 displayed an obviously faster growth than the control strain; the control strain pET32a barely survived (Fig. 6b). Under drought stress, *E. coli* strain pET32a showed a slightly reduced growth rate compared with *E. coli* strain pET32a-BplLTP1 (Fig. 6c). However, under alkali stress, the growth curve assay showed no significant difference between *E. coli* strains pET32a-BplLTP1 and pET32a, with maximum OD_{600} values of <0.3 for both strains (Fig. 6d).

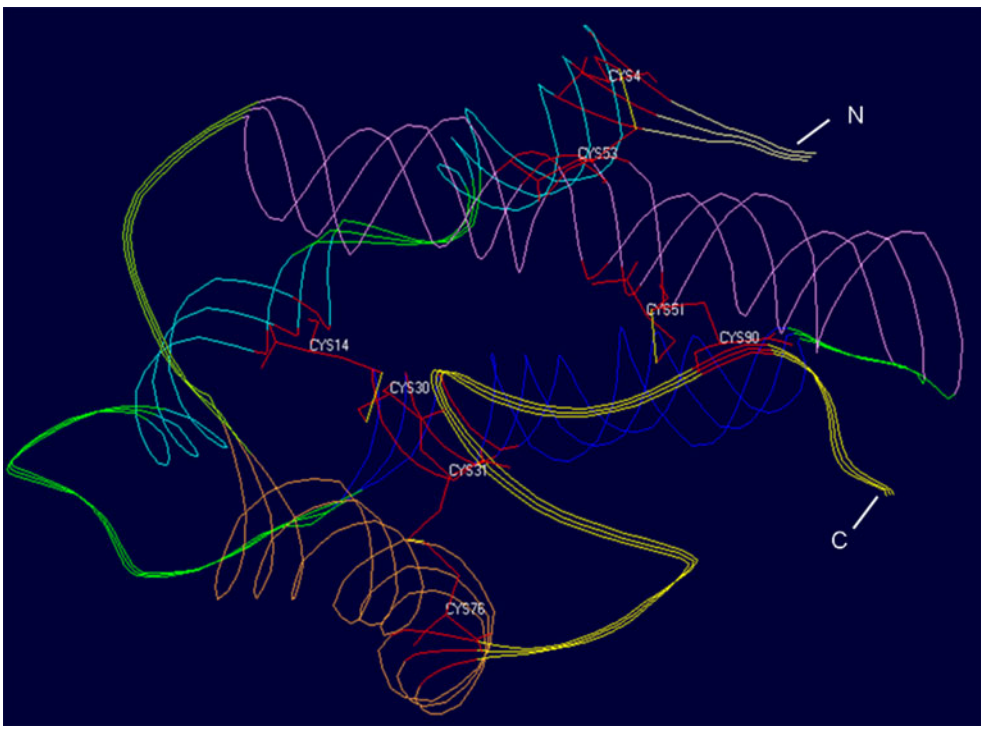

Fig. 3 A 3D model of putative BplLTP1 protein in three-wire representation. Cysteine residues involved in the four disulphide bridges are shown in *red*

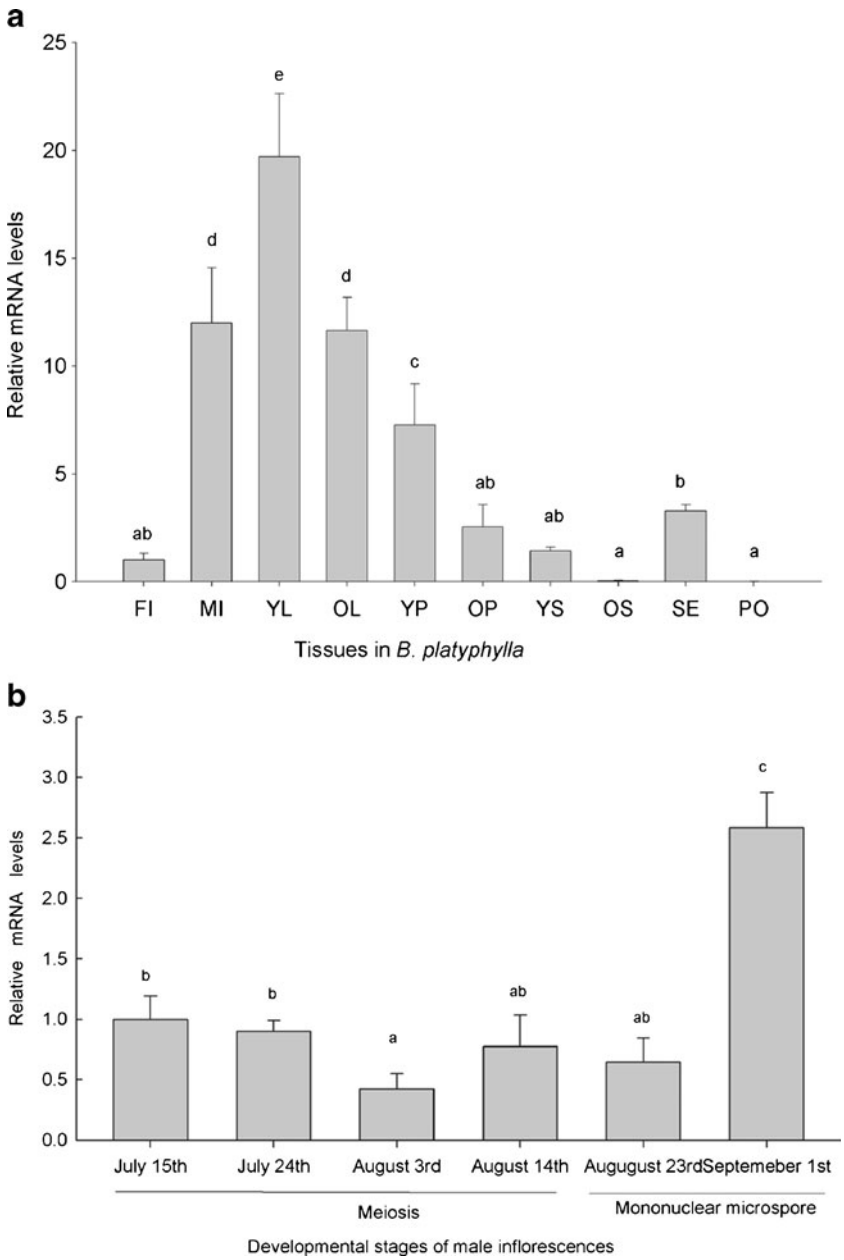

Fig. 4 qRT-PCR expression patterns of *BplLTP1* in *B. platyphylla*. **a** Expression analysis of *BplLTP1* in female inflorescences (*FI*), male inflorescences (*MI*), young leaves (*YL*), older leaves (*OL*), young petioles (*YP*), older petioles (*OP*), young stems (*YS*), older stems (*OS*), seeds (*SE*), and mature pollen (*PO*) of *B. platyphylla*. **b** Expression analysis of *BplLTP1* in different developmental stages of male inflorescences. The sampling dates of male inflorescences are indicated in the figure. Values represent the means of three replicate experiments with standard error. *Lowercase letters* indicate statistically significant differences ($P<0.05$)

Discussion

RACE is a simple but effective technique that has recently been employed to obtain full-length c-DNA sequences based on the sequences of known ESTs (Li et al. 2012; Phillips et al. 2013; Yang et al. 2012; Qin et al. 2013). In the present study, *BplLTP1* was successfully isolated from *B. platyphylla* using the RACE method. Similar to other nsLTPs, *BplLTP1* encodes a small protein with eight strictly conserved cysteine residues predicted to form four disulphide bridges, as well as a 26-amino acid N-terminal signal peptide predicted to target proteins to the secretory pathway (Carvalho and Gomes 2007). The molecular mass of mature BplLTP1 is estimated to be approximately 9.5 kDa, suggesting that this protein belongs to the type I LTP family (Carvalho and Gomes 2007).

The hypothetical 3D structure of BplLTP1 is compact and globular, comprising four α-helices and a C-terminal tail (Fig. 3). This structure forms a flexible hydrophobic pocket with a unique capacity to accommodate fatty acids, acyl-CoA, and phospholipids (Carvalho and Gomes 2007). In addition, the conserved in vitro lipid-binding motifs DRQ and CGV (Botton et al. 2002) were also observed in BplLTP1 and homologs from other plants (Fig. 1). These results indicate that BplLTP1 may possess lipid transfer activity. Although the cavities of nsLTPs are thought to be vitally important for the biological functions of these proteins, no direct evidence has revealed the function of these

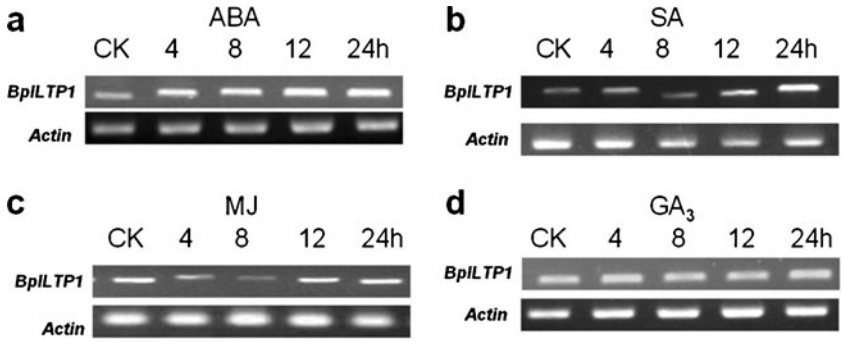

Fig. 5 Expression patterns of *BplLTP1* in response to exogenous hormone treatments. Expression analyses of *BplLTP1* were performed with total RNA extracted from leaves treated with abscisic acid (*ABA*) (**a**), salicylic acid (*SA*) (**b**), methyl jasmonate (*MJ*) (**c**), or gibberellin A (GA_3) (**d**). The *Actin* gene was used as an amplification and loading control. The number of PCR cycles in each experiment was 26. Experiments were repeated three times. A representative experiment is shown in the figure

cavities in vivo (Yeats and Rose 2008). Blein et al. (2002) found that nsLTP from wheat (*Triticum aestivum*) cannot load lipids from intact membranes under normal physiological conditions.

In a variety of plants, nsLTP genes have different transcription patterns in diverse tissues during various developmental stages and under different physiological conditions (Carvalho and Gomes 2007). For example, nsLTPs have been found in vascular tissues and epidermal cells during the formative stage of *Arabidopsis* development (Thoma et al. 1994) as well as in the cuticular waxy layer of broccoli (Pyee et al. 1994). The expression patterns of *BplLTP1* demonstrated that this gene is expressed at higher levels in young tissues (including young leaves, young stems, and young petioles) than in older tissues

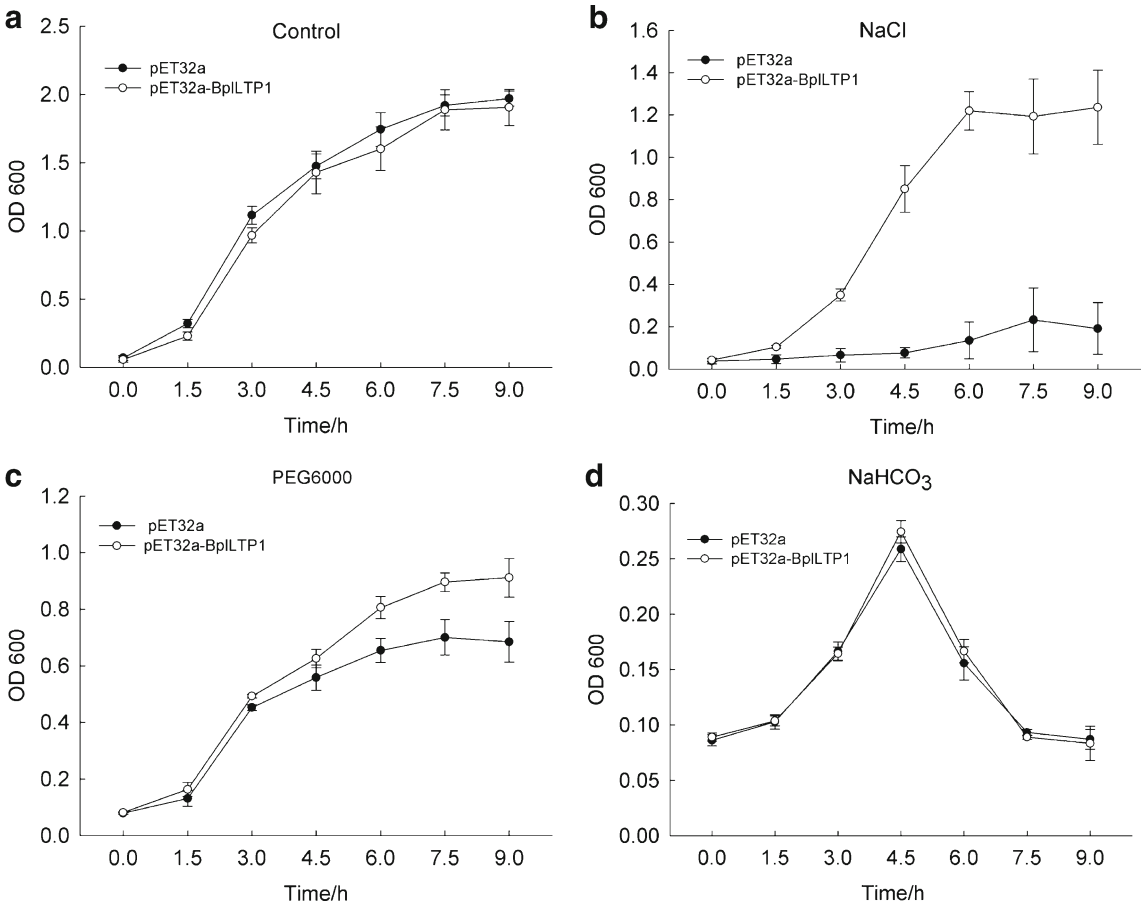

Fig. 6 Growth curves of *E. coli* BL21 (DE3) strains harboring recombinant plasmid pET32a-BplLTP1 or empty vector pET32a(+). Growth curves of *E. coli* BL21 (DE3) strains in liquid LB medium (100 $\mu g ml^{-1}$ ampicillin) with no supplements (control) (**a**) or supplemented with 0.4 M NaCl (**b**), 20 % (*w*/*v*) PEG 6000 (**c**), or 0.2 M $NaHCO_3$ (**d**). Experiments were repeated three times. Data represent the mean ± SE

(including older leaves, older stems, and older petioles; Fig. 4a). This expression pattern suggests that *BplLTP1* plays a necessary role in the formation and deposition of cuticular material (Pyee et al. 1994; Thoma et al. 1994). Similar results were also obtained in previous studies of plant nsLTPs. For instance, immunological measurements showed that nsLTP from broccoli is highly expressed in young leaves, constituting 50 % of leafy proteins, while the level of expression drops to only 4 % when the leaves age (Pyee et al. 1994). The *nsltp1* gene from *Arabidopsis* is expressed at high levels in young developing tissues, and its expression declines in fully expanded tissues; this pattern is also observed in the petal and sepal abscission zones (Thoma et al. 1994).

It is noteworthy that the expression level of *BplLTP1* in male inflorescences was up to 12-fold that in female inflorescences (Fig. 4a). We measured the expression levels of *BplLTP1* in male inflorescences at different developmental stages. Our results demonstrated that the expression of *BplLTP1* decreased during the meiosis stages, while the expression of this gene was significantly increased during the mononuclear microspore developmental stages (Fig. 4b; Liu and Yang 2006). These results suggest that *BplLTP1* plays an important role in male inflorescences. Unfortunately, we cannot determine the direct link between *BplLTP1* and pollen development from these data because the male inflorescences contained abundant bracts. Nevertheless, our results indirectly suggest that *BplLTP1* plays a role during pollen development (Zhang et al. 2010; Chen et al. 2011). A previous qRT-PCR analysis showed that *OsC6* expression is increased in anthers, beginning at the early mononuclear microspore development stage, while *OsC6* transcripts are barely detectable during the formation of mature trinucleate pollen (Zhang et al. 2010). This expression pattern, which is similar to the expression pattern of *BplLTP1*, helps explain why *BplLTP1* was almost undetectable in mature pollen (Fig. 4a).

NsLTPs are responsive to various plant hormones (Wu et al. 2004; Wang et al. 2009; Jung et al. 2006; Yubero-Serrano et al. 2003). ABA is considered to be a stress hormone because of its important role in plant stress responses. There is much overlap in the expression patterns of stress-related genes after plants are exposed to drought, high salt, or exogenous ABA (Zhang et al. 2006; Leung and Giraudat 1998). Previous studies have shown that several putative *cis*-acting elements, such as ABRE, DRE, G-box, and coupling elements, which are required for ABA-induced gene expression, are not found in the promoter region of LTP (Jung et al. 2006; George and Parida 2010). However, in this study, the transcription of *BplLTP1* was strongly induced by exogenous ABA treatment (Fig. 5a), suggesting that ABA upregulates LTP gene expression through the action of other elements rather than by direct contact with the promoter of this gene. SA and MJ are also plant signaling molecules involved in stress responses. Many studies have shown that the SA- and JA-mediated signaling pathways interact in an antagonistic manner (Niki et al. 1998; Kachroo et al. 2001). *BplLTP1* expression was induced in leaves treated with exogenous SA (Fig. 5b), but suppressed by exogenous MJ treatment (Fig. 5c). These results suggest that MJ might antagonize the induction of *BplLTP1* by the stress signaling molecule SA. In this study, we found that exogenous GA_3 treatment did not increase *BplLTP1* transcription in the leaves of *B. platyphylla*. This result suggests that GA_3 is not an effective signaling molecule for the induction of *BplLTP1* transcription.

Prokaryotic expression systems are an effective way to produce exogenous protein. Previous studies have shown that the expression of exogenous plant genes can directly contribute to increasing stress tolerance in the host bacterium (Yamada et al. 2002; Miyasaka et al. 2000; Lan et al. 2005). In this study, to elucidate the contribution of *BplLTP1* to abiotic stress responses, recombinant *E. coli* harboring *BplLTP1* was subjected to abiotic stresses including salt, alkali, and drought stress. Previous studies have shown that nsLTP expression is induced under salt stress conditions (Jang et al. 2004; Jung et al. 2003; Wu et al. 2004; Wang et al. 2009). Furthermore, overexpression of the *CALTP* gene enhances salt stress resistance in transgenic *Arabidopsis* (Jung et al. 2005). These results indicate that nsLTPs play an important role in salt stress response. In this study, our results demonstrated that the expression of *BplLTP1* increases salt stress tolerance in the host *E. coli*, suggesting that *BplLTP1* plays a role in plant salt tolerance response. The induction of nsLTP by drought stress occurs in wheat (*T. aestivum*; Jang et al. 2004), pepper (*Capsicum annuum*; Jung et al. 2003), bromegrass (*Bromus inermis*; Wu et al. 2004), and tamarix (*Tamarix hispida*; Wang et al. 2009). In addition, the overexpression of the nsLTP gene increases drought tolerance in transgenic *Arabidopsis* (Jung et al. 2005). The expression patterns of *BplLTP1* suggest that this gene plays a role in the formation and deposition of cuticular material (Pyee et al. 1994; Thoma et al. 1994). Epicuticular wax plays a significant physiological role in maintaining water balance in plants (Lemieux 1996). Our data also indicate that the expression of *BplLTP1* in host *E. coli* can increase drought stress tolerance. Taken together, these results suggest that *BplLTP1* plays a role in plant drought tolerance. Moreover, the upregulation of *BplLTP1* expression in response to the exogenous application of the plant hormones ABA and SA may contribute to plant survival by increasing cuticle thickness in response to stress (Jung et al. 2003). Liu et al. (2008) found that the expression of *Polygonum sibiricum* nsLTPs (PsnsLTPs) is significantly increased under $NaHCO_3$ stress, which suggests that PsnsLTPs play an important role in saline stress resistance. However, our results indicate that the alkali resistance of host *E. coli* is not increased by the expression of BplLTP1. Whether this is also the case in *B. platyphylla* remains to be determined.

Acknowledgments The work was supported by grants from the National Natural Science Foundation of China (31100449) and the Key Project of Chinese Ministry of Education (109053).

References

Ariizumi TA, Amagai MA, Shibata DS, Hatakeyama KH, Watanabe MW, Toriyama KT (2002) Comparative study of promoter activity of three anther-specific genes encoding lipid transfer protein, xyloglucan endotransglucosylase/hydrolase and polygalacturonase in transgenic *Arabidopsis thaliana*. Plant Cell Rep 21:90–96. doi:10.1007/s00299-002-0487-3

Arnold K, Bordoli L, Kopp J, Schwede T (2006) The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics 22:195–201. doi:10.1093/bioinformatics/bti770

Arondel V, Vergnolle C, Cantrel C, Kader J-C (2000) Lipid transfer proteins are encoded by a small multigene family in *Arabidopsis thaliana*. Plant Sci 157:1–12. doi:10.1016/s0168-9452(00)00232-6

Bjellqvist B, Hughes GJ, Pasquali C, Paquet N, Ravier F, Sanchez J-C, Frutiger S, Hochstrasser D (1993) The focusing positions of polypeptides in immobilized pH gradients can be predicted from their amino acid sequences. Electrophoresis 14:1023–1031. doi:10.1002/elps.11501401163

Blein JP, Coutos-Thévenot P, Marion D, Ponchet M (2002) From elicitins to lipid-transfer proteins: a new insight in cell signalling involved in plant defence mechanisms. Trends Plant Sci 7:293–296. doi:10.1016/s1360-1385(02)02284-7

Botton A, Begheldo M, Rasori A, Bonghi C, Tonutti P (2002) Differential expression of two lipid transfer protein genes in reproductive organs of peach (*Prunus persica* L. Batsch). Plant Sci 163:993–1000. doi:10.1016/s0168-9452(02)00271-6

Carvalho AO, Gomes VM (2007) Role of plant lipid transfer proteins in plant cell physiology—a concise review. Peptides 28:1144–1153. doi:10.1016/j.peptides.2007.03.004

Chae K, Kieslich CA, Morikis D, Kim S-C, Lord EM (2009) A gain-of-function mutation of *Arabidopsis* lipid transfer protein 5 disturbs pollen tube tip growth and fertilization. Plant Cell 21:3902–3914. doi:10.1105/tpc.109.070854

Chang S, Puryear J, Cairney J (1993) A simple and efficient method for isolating RNA from pine trees. Plant Mol Biol Rep 11:113–116. doi:10.1007/bf02670468

Chen C, Chen G, Hao X, Cao B, Chen Q, Liu S, Lei J (2011) CaMF2, an anther-specific lipid transfer protein (LTP) gene, affects pollen development in *Capsicum annuum* L. Plant Sci 181:439–448. doi:10.1016/j.plantsci.2011.07.003

Choi YE, Lim S, Kim H-J, Han JY, Lee M-H, Yang Y, Kim J-A, Kim Y-S (2012) Tobacco NtLTP1, a glandular-specific lipid transfer protein, is required for lipid secretion from glandular trichomes. Plant J 70:480–491. doi:10.1111/j.1365-313X.2011.04886.x

de Oliveira Carvalho A, Teodoro CES, Da Cunha M, Okorokova-Façanha AL, Okorokov LA, Fernandes KVS, Gomes VM (2004) Intracellular localization of a lipid transfer protein in *Vigna unguiculata* seeds. Physiol Plant 122:328–336. doi:10.1111/j.1399-3054.2004.00413.x

DeBono A, Yeats TH, Rose JKC, Bird D, Jetter R, Kunst L, Samuels L (2009) *Arabidopsis* LTPG is a glycosylphosphatidylinositol-anchored lipid transfer protein required for export of lipids to the plant surface. Plant Cell 21:1230–1238. doi:10.1105/tpc.108.064451

Gao G, Jin LP, Xie KY, Qu DY (2009) The potato *StLTPa7* gene displays a complex Ca^{2+}-associated pattern of expression during the early stage of potato–*Ralstonia solanacearum* interaction. Mol Plant Pathol 10:15–27. doi:10.1111/j.1364-3703.2008.00508.x

George S, Parida A (2010) Characterization of an oxidative stress inducible nonspecific lipid transfer protein coding cDNA and its promoter from drought tolerant plant *Prosopis juliflora*. Plant Mol Biol Rep 28:32–40. doi:10.1007/s11105-009-0127-y

Jang CS, Lee HJ, Chang SJ, Seo YW (2004) Expression and promoter analysis of the *TaLTP1* gene induced by drought and salt stress in wheat (*Triticum aestivum* L.). Plant Sci 167:995–1001. doi:10.1016/j.plantsci.2004.05.019

José-Estanyol M, Gomis-Rüth FX, Puigdomènech P (2004) The eight-cysteine motif, a versatile structure in plant proteins. Plant Physiol Biochem 42:355–365. doi:10.1016/j.plaphy.2004.03.009

Jung HW, Kim W, Hwang BK (2003) Three pathogen-inducible genes encoding lipid transfer protein from pepper are differentially activated by pathogens, abiotic, and environmental stresses. Plant Cell Environ 26:915–928. doi:10.1046/j.1365-3040.2003.01024.x

Jung HW, Kim KD, Hwang BK (2005) Identification of pathogen-responsive regions in the promoter of a pepper lipid transfer protein gene (*CALTPI*) and the enhanced resistance of the CALTPI transgenic *Arabidopsis* against pathogen and environmental stresses. Planta 221:361–373

Jung HW, Lim CW, Hwang BK (2006) Isolation and functional analysis of a pepper lipid transfer protein III (*CALTPIII*) gene promoter during signaling to pathogen, abiotic and environmental stresses. Plant Sci 170:258–266. doi:10.1016/j.plantsci.2005.08.010

Kachroo P, Shanklin J, Shah J, Whittle EJ, Klessig DF (2001) A fatty acid desaturase modulates the activation of defense signaling pathways in plants. Proc Natl Acad Sci U S A 98:9448–9453

Kader JC (1996) Lipid-transfer proteins in plants. Annu Rev Plant Physiol Plant Mol Biol 47:627–654

Kader JC (1997) Lipid-transfer proteins: a puzzling family of plant proteins. Trends Plant Sci 2:66–70. doi:10.1016/s1360-1385(97)82565-4

Kader JC, Julienne M, Vergnolle C (1984) Purification and characterization of a spinach-leaf protein capable of transferring phospholipids from liposomes to mitochondria or chloroplasts. Eur J Biochem 139:411–416

Knight H, Knight MR (2001) Abiotic stress signalling pathways: specificity and cross-talk. Trends Plant Sci 6:262–267. doi:10.1016/s1360-1385(01)01946-x

Lan Y, Cai D, Zheng YZ (2005) Expression of three differen t group soybean lea genes enhanced stress tolerance in *Escherichia coli*. J Integr Plant Biol 47:613–621

Lee SB, Go YS, Bae H-J, Park JH, Cho SH, Cho HJ, Lee DS, Park OK, Hwang I, Suh MC (2009) Disruption of glycosylphosphatidylinositol-anchored lipid transfer protein gene altered cuticular lipid composition, increased plastoglobules, and enhanced susceptibility to infection by the fungal pathogen *Alternaria brassicicola*. Plant Physiol 150:42–54. doi:10.1104/pp.109.137745

Lemieux B (1996) Molecular genetics of epicuticular wax biosynthesis. Trends Plant Sci 1:312–318

Leung J, Giraudat J (1998) Abscisic acid signal transduction. Annu Rev Plant Physiol Plant Mol Biol 49:199–222

Li Q-F, Sun S, Yuan D-Y, Yu H-X, Gu M-H, Liu Q-Q (2010) Validation of candidate reference genes for the accurate normalization of real-time quantitative RT-PCR data in rice during seed development. Plant Mol Biol Rep 28:49–57. doi:10.1007/s11105-009-0124-1

Li S, Yu F, Wang M, Guo X, Li H (2012) Molecular characterization of a *Nicotiana tabacum* NtRDR6 gene. Plant Mol Biol Rep 30:1375–1384. doi:10.1007/s11105-012-0455-1

Liu X, Yang C (2006) Temporal characteristics of developmental cycles of female and male flowers in *Betula platyphylla* in northeastern China. Sci Silvae Sin 42:28–32

Liu GJ, Tian X, Liu CC, Qu CP, Liu GF, Yang CP (2008) Cloning of coding sequence of non-specific transfer protein from *Polygonum*

sibiricum Laxm. and expression under salinity stress. Chin J Biochem Mol Biol 24:1140–1145

Lloyd G, McCown B (1980) Commercially-feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. Proc Int Plant Prop Soc 3:421–427

Mahajan S, Tuteja N (2005) Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444:139–158. doi:10.1016/j.abb.2005.10.018

Marchler-Bauer A, Lu S, Anderson JB, Chitsaz F, Derbyshire MK, DeWeese-Scott C, Fong JH, Geer LY, Geer RC, Gonzales NR, Gwadz M, Hurwitz DI, Jackson JD, Ke Z, Lanczycki CJ, Lu F, Marchler GH, Mullokandov M, Omelchenko MV, Robertson CL, Song JS, Thanki N, Yamashita RA, Zhang D, Zhang N, Zheng C, Bryant SH (2011) CDD: a conserved domain database for the functional annotation of proteins. Nucleic Acids Res 39:225–229. doi:10.1093/nar/gkq1189

Miyasaka H, Kanaboshi H, Ikeda K (2000) Isolation of several anti-stress genes from the halotolerant green alga *Chlamydomonas* by simple functional expression screening with *Escherichia coli*. World J Microbiol Biotechnol 16:23–29. doi:10.1023/a:1008982332139

Niki T, Mitsuhara I, Seo S, Ohtsubo N, Ohashi Y (1998) Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. Plant Cell Physiol 39:500–507

Pasquato N, Berni R, Folli C, Folloni S, Cianci M, Pantano S, Helliwell JR, Zanotti G (2006) Crystal structure of peach Pru p3, the prototypic member of the family of pant non-specific lipid transfer protein pan-allergens. J Mol Biol 356:684–694. doi:10.1016/j.jmb.2005.11.063

Petersen TN, Brunak S, von Heijne G, Nielsen H (2011) SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat Methods 8:785–786, http://www.nature.com/nmeth/journal/v8/n10/abs/nmeth.1701.html#supplementary-information

Phillips SM, Dubery IA, Heerden H (2013) Identification and molecular characterisation of a lectin receptor-like kinase (*GhLecRK-2*) from cotton. Plant Mol Biol Rep 31:9–20. doi:10.1007/s11105-012-0470-2

Pyee J, Yu HS, Kolattukudy PE (1994) Identification of a lipid transfer protein as the major protein in the surface wax of broccoli (*Brassica oleracea*) leaves. Arch Biochem Biophys 311:460–468. doi:10.1006/abbi.1994.1263

Qi J, Yu S, Zhang F, Shen X, Zhao X, Yu Y, Zhang D (2010) Reference gene selection for real-time quantitative polymerase chain reaction of mRNA transcript levels in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). Plant Mol Biol Rep 28:597–604. doi:10.1007/s11105-010-0185-1

Qin Q, Kaas Q, Zhang L, Xu K, Li N, Zheng W, Lai Q (2013) Isolation and characterization of a cytosolic pyruvate kinase cDNA from loquat (*Eriobotrya japonica* Lindl.). Plant Mol Biol Rep 31:109–119. doi:10.1007/s11105-012-0479-6

Samuel D, Liu Y-J, Cheng C-S, Lyu P-C (2002) Solution structure of plant nonspecific lipid transfer protein-2 from rice (*Oryza sativa*). J Biol Chem 277:35267–35273. doi:10.1074/jbc.M203113200

Sarowar S, Kim YJ, Kim KD, Hwang BK, Ok SH, Shin JS (2008) Overexpression of lipid transfer protein (LTP) genes enhances resistance to plant pathogens and LTP functions in long-distance systemic signaling in tobacco. Plant Cell Rep 28:419–427. doi:10.1007/s00299-008-0653-3

Shin DH, Lee JY, Hwang KY, Kyu Kim K, Suh SW (1995) High-resolution crystal structure of the non-specific lipid-transfer protein from maize seedlings. Structure 3:189–199. doi:10.1016/s0969-2126(01)00149-6

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Thoma S, Kaneko Y, Somerville C (1993) A non-specific lipid transfer protein from *Arabidopsis* is a cell wall protein. Plant J 3:427–436. doi:10.1046/j.1365-313X.1993.t01-25-00999.x

Thoma S, Hecht U, Kippers A, Botella J, De Vries S, Somerville C (1994) Tissue-specific expression of a gene encoding a cell wall-localized lipid transfer protein from *Arabidopsis*. Plant Physiol 105:35–45. doi:10.1104/pp.105.1.35

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Wang C, Yang C, Gao C, Wang Y (2009) Cloning and expression analysis of 14 lipid transfer protein genes from *Tamarix hispida* responding to different abiotic stresses. Tree Physiol 29:1607–1619. doi:10.1093/treephys/tpp082

Wu G, Robertson AJ, Liu X, Zheng P, Wilen RW, Nesbitt NT, Gusta LV (2004) A lipid transfer protein gene BG-14 is differentially regulated by abiotic stress, ABA, anisomycin, and sphingosine in bromegrass (*Bromus inermis*). J Plant Physiol 161:449–458. doi:10.1078/0176-1617-01259

Xing L, Liu X-m (2011) Characterization of *Betula platyphylla* gene transcripts associated with early development of male inflorescence. Mol Biol Rep 39:929–935. doi:10.1007/s11033-011-0818-y

Yamada M (1992) Lipid transfer proteins in plants and microorganisms. Plant Cell Physiol 33:1–6

Yamada A, Sekiguchi M, Mimura T, Ozeki Y (2002) The role of plant CCTα in salt- and osmotic-stress tolerance. Plant Cell Physiol 43:1043–1048. doi:10.1093/pcp/pcf120

Yang Y, Xian Z, Wu Y, Li J, Deng W (2012) Molecular cloning and expression analysis of a putative 5′–3′ EXORIBONUCLEASE4 (XRN4) gene from tomato. Plant Mol Biol Rep 30:1348–1356. doi:10.1007/s11105-012-0449-z

Yeats TH, Rose JK (2008) The biochemistry and biology of extracellular plant lipid-transfer proteins (LTPs). Protein Sci 17:191–198

Yubero-Serrano EM, Moyano E, Medina-Escobar N, Muñoz-Blanco J, Caballero JL (2003) Identification of a strawberry gene encoding a non-specific lipid transfer protein that responds to ABA, wounding and cold stress. J Exp Bot 54:1865–1877. doi:10.1093/jxb/erg211

Zhang JH, Jia WS, Yang JC, Ismail AM (2006) Role of ABA in integrating plant responses to drought and salt stresses. Field Crop Res 97:111–119

Zhang D, Liang W, Yin C, Zong J, Gu F (2010) *OsC6*, encoding a lipid transfer protein, is required for postmeiotic anther development in rice. Plant Physiol 154:149–162. doi:10.1104/pp.110.158865