Characterization of Thaumatin-like Gene Family and Identification of *Pectobacterium carotovorum* subsp. *carotovorum* Inducible Genes in *Brassica oleracea*

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ABSTRACT Cabbage (*Brassica oleracea*) is a very important vegetable worldwide and biotic stress is a crucial issue for this crop. Enhancement of resistance by exploiting stress resistance-related genes offers the most efficient approach to address this issue. Among the stress resistance-related genes, thaumatin-like proteins (TLPs) play a vital role in enhancement of resistance against stresses. In this study, we identified 12 TLPs from *B. oleracea* genomic DNA sequencing database, analyzed their sequences and compared with other published pathogenesis-related TLPs, and found a high degree of homology with them. In addition, these genes showed an organ-specific expression, three of which expressed differentially after *Pectobacterium carotovorum* subsp. *carotovorum* infection in cabbage plants. Data obtained in this study suggest the probable involvement of TLPs in resistance against soft rot disease of *Brassica*.

Keywords *Brassica*, Gene expression, *Pectobacterium carotovorum* subsp. *carotovorum*, Thaumatin-like protein

INTRODUCTION

Brassica crops are sternly challenged by various fungal, bacterial, and viral diseases and insect pests which led to enormous losses of yield. *Brassica* vegetables are highly susceptible to bacterial soft rot disease, which is caused by a gram-negative bacterium, *Pectobacterium carotovorum* subsp. *carotovorum* (*Pcc*). Bacterial soft rot is the most severe and destructive disease, which causes serious damage and economic losses across the members of *Brassica*. Control of this pathogen is difficult due to its ability to infect a wide range of hosts and to survive on plant debris. Effective chemical controls are not available so far. Cultural practices, including raised planting beds, reduced plant density, and delayed planting dates can reduce disease frequency and progression, but may reduce agricultural efficiency (Fritz and Honma 1987). Genetic tolerance or resistance may represent an ideal alternative approach. But the traditional recurrent selection procedure is labor intensive

and time-consuming. Moreover, due to lack of natural resistant genes against soft rot disease, the proceeding of routine raising disease-resistance breeding is restricted (Li 1995). Therefore, genetic engineering of soft rot disease tolerance in *Brassica* is of significant interest to agricultural biotechnology.

Plants respond to biotic stress factors by producing various defense-related proteins such as pathogenesis-related (PR) proteins, hydroxyproline-rich glycoproteins (HRGPs), and several key enzymes involved in the synthesis of phenylpropanoids, phytoalexins and lignins (Collinge and Slusarenko 1987; Bowles 1990; Dixon and Lamb 1990). One promising strategy to develop resistance against biotic stresses is based on the exploitation of PRs and other stress responsive genes in transgenic plants. Accumulation of PRs extends into non-inoculated plant parts that, upon challenge, exhibit acquired resistance (Van Loon and Van Kammen 1970; Ryals et al. 1996). PR proteins are classified into 17 families based on sequence similarity and biochemical

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functions (Van loon et al. 2006). An important common feature of most PR proteins is their antifungal effect. In addition, some PR proteins exhibit antibacterial, insecticidal, nematocidal, and anti-viral action (Van Loon and Van Strien 1999; Selitrennikoff 2001; Van Loon 2001).

Owing to the sequence homology between PR-5 proteins and thaumatin, an intensely sweet-tasting protein isolated from the fruit of the West African rainforest shrub *Thaumatococcus daniellii* (Bennett) Benth (Iyengar et al. 1979), members of this family of proteins are referred to as thaumatin-like proteins (TLPs). This family also includes the closely related proteins permatin, osmotin and zeamatin. TLPs play a variety of roles including development of seed, fruit and flower tissues, and specific TLPs have been shown to protect plants against osmotic stress, pathogen attack and the deleterious effects of freezing (Velazhahan et al. 1999; An lovar and Dermastia 2003). The expression of some TLPs is induced by biotic and abiotic factors, such as microbial infection, osmotic stress, abscisic acid (ABA), ethylene, salicylic acid (SA), methyl jasmonate (MJ) and elicitors (Kitajima and Sato 1999; Velazhahan et al. 1999). Purified TLPs from several plant species have *in vitro* anti-fungal activity. Several antifungal modes of action have been described such as membrane permeabilization (Vigers et al. 1991), β -glucan binding and degradation (Sakamoto et al. 2006), inhibition of enzymes such as xylanases (Fierens et al. 2007), α -amylase, or trypsin (Schimoler-O'Rourke et al. 2001), as well as an apoptosis-inducing mechanism reported in yeast (Narasimhan et al. 2005). A PR5 protein from *Arabidopsis thaliana* ATLP3, showed considerable antifungal activity against pathogenic fungi such as *Verticillium alboatrum*, *V. dahliae* and *Fusarium oxysporum* (Hu and Ready 1997). PR5 proteins from mutant barley grain inhibited growth of *Trichoderma viride* and *Candida albicans* (Hejgaard et al. 1991). In addition to inhibiting hyphal growth and spore germination, some TLPs greatly increase the permeability of fungal and oomycete plasma membranes (Yun et al. 1997; Kitajima and Sato 1999). Overexpression of TLPs appears to enhance resistance to fungal disease (Liu et al. 1994; Zhu et al. 1996; Chen et al. 1999). Moreover, TLPs in various plant-derived foods (Hoffmann-Sommergruber 2002) and conifer pollen (Midoro-Horiuti et al. 2000; Cortegano et al.

2004) are allergens.

In our previous study, we characterized 20 thaumatin-like genes of *B. rapa* and found four genes to respond after *P. carotovorum* subsp. *carotovorum* infection in Chinese cabbage (Ahmed et al. 2013). In relation to the previous study, we identified 12 thaumatin-like genes of *B. oleracea* from *Brassica oleracea* Genome Database (Bolbase) (<http://119.97.203.210/bolbase/index.html>). We then analyzed the sequences and studied their homology with pathogenesis-related thaumatin-like proteins of other species. In addition, expression of these genes was analyzed in different organs and after infection with *P. carotovorum* subsp. *carotovorum* in cabbage plants. We also discussed their association with biotic stress resistance in *Brassica*.

MATERIALS AND METHODS

Plant materials

Cabbage (*Brassica oleracea* 'ASC621') plants were grown at the Department of Horticulture, Sunchon National University, Korea. Fresh roots, stems, leaves and flower buds of the Chinese cabbage were harvested, immediately frozen in liquid nitrogen, and then stored at -80°C until subsequent analysis in an organ-specific expression study.

Stress treatments

Chinese cabbage (*B. oleracea* 'ASC621') plants were grown for 6 weeks under culture room conditions with 16 h light and 8 h dark maintaining 25°C temperature prior to treatment. The *P. carotovorum* subsp. *carotovorum* stock ($10\ \mu\text{l}$) was cultured in 25 ml of liquid YEP medium until OD_{600} reading is 1.4 or 1,170, cfu ml^{-1} and then diluted it to $\text{OD}_{600} = 1.19$ or 1×10^6 CFU ml^{-1} by adding double-distilled water. For pathogen inoculation, $10\ \mu\text{l}$ of *P. carotovorum* subsp. *carotovorum* culture solution (1×10^6 cfu ml^{-1}) was added to the freshly needle-wounded site (at the lower 1/3 position) of the midrib of the upper third leaves, and incubated at 25°C covering with polyvinyl bags to maintain 80-90% humidity. All inoculations were performed three times, and the infection was confirmed by observing disease lesion in the leaves of Chinese cabbage plants. About one-third part from the top of the infected leaves were harvested for RNA

extraction at 0, 6, 12, 24, 48 and 72 h after inoculation, frozen in liquid nitrogen, and stored at -80°C.

RNA extraction

Total RNA of previously frozen roots, stems, leaves, flower buds and stress treated samples with Rneasy mini kit (Qiagen, USA) was extracted and treated with RNase-free DNase (Promega, USA) to remove genomic DNA contaminants. The cDNA was subsequently synthesized using a Superscript[®] III First-Strand synthesis kit (Invitrogen, USA) according to the manufacturer's instructions.

Sequence analysis

We collected 12 thaumatin-like proteins (*BoTLP1* to *12*) from *Brassica oleracea* Genome Database (Bolbase) (<http://119.97.203.210/bolbase/index.html>) by blast searching of each 17 *B. oleracea* thaumatin-like protein ESTs (Table 1) collected from cabbage genomic project database ([http://210.219.43.182:8080/brdb/search.jsp?species=bol &classes=all&keyword=BOLS24099&length=0&identit y=0&block=all#](http://210.219.43.182:8080/brdb/search.jsp?species=bol&classes=all&keyword=BOLS24099&length=0&identit y=0&block=all#)). The primary structure of genes was analyzed using protParam (<http://expasy.org/tools/protparam.html>) and Augustus (<http://augustus.gobics.de/submission>). An alignment search was conducted using NCBI BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>) and the program

BLASTp, with the “nr” database. Typical domains were analyzed using the EMBL web tool (http://smart.embl.de/smart/set_mode.cgi?GENOMIC=1). Multiple protein sequences were aligned using PIR (<http://pir.georgetown.edu/pirwww/search/multialn.shtml>), and a phylogenetic tree was constructed according to the neighbor-joining method using the MEGA4.1 software (<http://www.megasoftware.net>) (Saitou and Nei 1987; Tamura et al. 2007).

Expression analysis

RT-PCR was conducted using an AMV one step RT-PCR kit (Takara, Japan). Specific primers for all the genes were used for RT-PCR, and actin primers of *Brassica rapa* (FJ969844) were used as control (Table 1). PCR was performed using 50 ng of cDNA from the roots, leaves, stems and flower buds as templates in master mixes composed of 20 pmol of each primer, 150 µM of each dNTP, 1.2 U of *Taq* polymerase, 1x *Taq* polymerase buffer, and double-distilled H₂O diluted to a total volume of 20 µl in 0.5 ml PCR tubes. The samples were then subjected to the following conditions: pre-denaturing at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 1 min, with a final extension for 5 min at 72°C. Real-time quantitative PCR was performed using 1 µl of cDNA in a

Table 1. Primer information of 12 thaumatin-like genes of *B. oleracea* for RT and Real time PCR.

Gene name	Acc. No. of ESTs	Primer sequence	
		Forward	Reverse
<i>BoTLP1</i>	BOLC06439	CAGAAATCAACAAGCTACCC	GATCCGTCTTGTAAACATCGT
<i>BoTLP2</i>	BOLC11620	TTTTCAACCATCCAGAATAGC	GTCGAAGTTACAGCCTGTTC
<i>BoTLP3</i>	BOLC12781	TCTACCGTTTTCAAGAGAGC	CCGTACGGATTCTGAGTTAG
<i>BoTLP4</i>	BOLC13543	CTACTCTCGCAGAGATCACC	GAGTTAAAGGCAGAACATGC
<i>BoTLP5</i>	BOLC14356	CCCTGAAAGAAGAATGAGAA	ACAAGACGAGCACCTACACT
<i>BoTLP6</i>	BOLC17729	ACGACTTACTCGATGACGTT	ATCTTCCGTACATAGCAAGC
<i>BoTLP7</i>	BOLC20473	CCGCAAAAGCTCTTTATTAC	TATAGCTACGCATACGACGA
<i>BoTLP8</i>	BOLC20492	ATCCAGATGAGAATCACGAC	ACAAGTCTCCGGTTTATTGA
<i>BoTLP9</i>	BOLC23111	GTAGAGACGGGAACAAAACA	AAAGTTGCAGTGAAAAGCTC
<i>BoTLP10</i>	BOLC23113	ACGGGAGGATTCTTAATTGT	CAACGCAGCATTAGATAACA
<i>BoTLP11</i>	BOLC23820	CGTCCGGATTAATACTTGAG	AGTTGTACACCGTTGTCTCC
<i>BoTLP12</i>	BOLS14849	AGCAAAATCCATATCACAGG	GACTGTCCCACATCAAGTTT
<i>Br-Actin</i>	FJ969844	CAACCAATCGTCTGTGACAA	ATGTCTTGGCCTACCAACAA

25 µl reaction volume employing iTaq™ SYBR® Green Super-mix with ROX (California, USA). The specific primers used for real-time PCR are listed in Table 1. The conditions for real-time PCR were as follows: 10 min at 95°C, followed by 40 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 45 s. The fluorescence was measured following the last step of each cycle, and three replications were used for each sample. Amplification, detection, and data analysis were conducted using a Rotor-Gene 6000 real-time rotary analyzer (Corbett Life Science, Australia).

RESULTS AND DISCUSSION

Identification and sequence analysis

In this study, 12 thaumatin-like proteins, designated *B. oleracea* Thaumatin-like Protein (BoTLP) were identified and their sequences were analyzed and presented in the Table 2. The size of the 12 BrTLPs varies from 133 to 428 amino acids (~14.66 to 45.33 kDa), and the predicted isoelectric points vary from 4.25 to 9.07. Analysis of the protein domain organization showed that all these proteins contained the characteristic thaumatin (THN) domain in the conserved region located mostly from 1 to 190 amino

acid positions of the protein sequences which covers almost 80 to 90% of the entire mature TLPs. Genomic DNA sequences pertaining structural information of all 12 genes were collected from *Brassica oleracea* Genome Database (Bolbase) (<http://119.97.203.210/bolbase/index.html>). Most typical TLPs described to date have a molecular weight ranging from 20 to 26 kDa, and generally possess 16 conserved cysteine residues that form eight disulfide bonds (Ghosh and Chakrabarti 2008). Several TLP structures have been solved so far, revealing a strongly conserved 3D organization with a characteristic acidic cleft domain that comprises the five highly conserved amino acids REDDD that are dispersed in the primary sequence (Koiwa et al. 1999). Despite good conservation of these amino acids in primary sequences, they do not organize into an acidic cleft at the 3D level (Vandermarliere et al. 2010). The BoTLPs also contained 16 cysteine residues, REDDD and FF hydrophobic motif conserved in an identical position with other published TLPs and sweet tasting thaumatin protein (Fig. 2A and B). Alongside, it has become evident that several families of cysteine-rich basic antimicrobial polypeptides play an important role in protecting plants against invading phytopathogens (Garcia-Olmedo et al. 1998). These sequence data revealed that the identified

Table 2. Overall analysis of thaumatin-like genes collected from the *Brassica oleracea* database.²⁾

Name of genes	Accession Number	ORF (bp)	Chromosome number	Protein			
				Length (aa)	THN domain Start and end	Mol.Wt. (KD)	pI
<i>BoTLP1</i>	Bol016062	1041	C03	346	1 to 184	35.58	4.25
<i>BoTLP2</i>	Bol044835	738	C08	245	1 to 185	25.32	5.05
<i>BoTLP3</i>	Bol022411	1098	C07	365	1 to 189	38.56	5.31
<i>BoTLP4</i>	Bol026250	738	C06	245	1 to 181	26.15	9.07
<i>BoTLP5</i>	Bol000789	825	Scaffold	274	1 to 185	29.22	8.91
<i>BoTLP6</i>	Bol026131	732	C06	243	1 to 180	25.63	4.62
<i>BoTLP7</i>	Bol026844	741	C05	246	1 to 184	25.46	4.83
<i>BoTLP8</i>	Bol044836	402	C08	133	1 to 72	14.66	8.29
<i>BoTLP9</i>	Bol027694	978	C06	325	1 to 185, trnsm 1	34.32	4.86
<i>BoTLP10</i>	Bol039281	963	C02	320	1 to 187	33.73	4.67
<i>BoTLP11</i>	Bol026846	747	C05	248	1 to 186	25.88	4.63
<i>BoTLP12</i>	Bol016440	1287	C02	428	1 to 190	45.33	4.95

²⁾ *Brassica oleracea* database (<http://119.97.203.210/bolbase/blast/blast.html>).

genes are thaumatin-like genes.

For comparison of these *BoTLPs* with other published TLPs, an alignment search was carried out using a BLAST search of the NCBI database. The deduced amino acid sequences of 12 *BoTLPs* shared high homology, primarily with pathogenesis-related TLPs of *A. thaliana* and some other homologous species (Table 3). We again retrieved 41 pathogenesis-related TLP sequences of different plant species from NCBI and a phylogenetic tree was constructed with the deduced amino acid sequences of 12 TLPs of *B. oleracea* using the NJ method (Fig. 1). In the phylogenetic

tree, 12 *BoTLPs* were divided into two main groups showing close relations with the PR TLP sequences of other plant species. Multiple alignment using PIR also revealed high similarity among the deduced amino acids of the TLPs of the two groups and the thaumatin protein of *T. daniellii* (Bennett) Benth (P02884), indicating their relatively conserved evolutionary relationship at the protein level (Fig. 2A and B). Thus, our results showed that the *BoTLP* genes showed high similarity with other PR TLPs and might play roles in defense activities against phytopathogens.

Table 3. Homology analysis of 12 thaumatin-like genes of *Brassica oleracea* ²⁾

Protein	Top matched clones	Top matched protein	Identity	Top homologous species	References
BoTLP1	NP568046	PR TLP ^{y)}	86%	<i>Arabidopsis thaliana</i>	Mayer et al. (1999)
	XP003540838	TLP	65%	<i>Glycine max</i>	Unpublished
BoTLP2	NP173365	PR TLP	72%	<i>A. thaliana</i>	Theologis et al. (2000)
	AEV57470	TLP1	70%	<i>Prunus persica</i>	Sherif et al. (2012)
BoTLP3	AED93335	PR TLP	71%	<i>A. thaliana</i>	Tabata et al. (2000)
	XP004309088	TLP1	71%	<i>Fragaria vesca</i> subsp. <i>vesca</i>	Unpublished
BoTLP4	ABV89611	PR TLP	99%	<i>B. rapa</i>	Ahmed et al. (2013)
	NP177503	PR TLP	96%	<i>A. thaliana</i>	Theologis et al. (2000)
BoTLP5	NP001078513	PR TLP	93%	<i>A. thaliana</i>	Mayer et al. (1999)
	XP003540840	TLP1	77%	<i>G. max</i>	Unpublished
BoTLP6	NP177640	TLP3	77%	<i>A. thaliana</i>	Theologis et al. (2000)
	AEV57470	TLP1	73%	<i>P. persica</i>	Sherif et al. (2012)
BoTLP7	NP173365	PR TLP	71%	<i>A. thaliana</i>	Theologis et al. (2000)
	BAD90814	TLP	66%	<i>Cryptomeria japonica</i>	Futamura et al. (2006)
BoTLP8	NP180054	PR TLP	74%	<i>A. thaliana</i>	Lin et al. (1999)
	XP003612186	TLP	64%	<i>Medicago truncatula</i>	Unpublished
BoTLP9	ABV89616	PR TLP	88%	<i>B. rapa</i>	Ahmed et al. (2013)
	NP177708	PR TLP	84%	<i>A. thaliana</i>	Theologis et al. (2000)
BoTLP10	ABV89616	PR TLP	98%	<i>B. rapa</i>	Ahmed et al. (2013)
	NP177708	PR TLP	85%	<i>A. thaliana</i>	Theologis et al. (2000)
BoTLP11	NP173365	PR TLP	67%	<i>A. thaliana</i>	Theologis et al. (2000)
	BAD90814	TLP	55%	<i>C. japonica</i>	Futamura et al. (2006)
BoTLP12	NP197850	PR TLP	79%	<i>A. thaliana</i>	Unpublished
	BAB11214	TLP	91%	<i>A. thaliana</i>	Sato et al. (1998)

²⁾ Analyzed using BLAST from NCBI, <http://www.ncbi.nlm.nih.gov/BLAST/>.

^{y)} PR TLP- Pathogenesis related thaumatin-like protein.

Organ-specific expression analysis

Gene expression in different organ of cabbage was carried out by RT-PCR and varying expression pattern was observed (Fig. 3). Specifically, *BoTLP1* and *10* showed expressions in all the organs tested except roots. *BoTLP2* and *8* showed high level of expression only in leaves, while *BoTLP3* showed expression in all organs except stem. *BoTLP4*, *7*, *9* and *12* expressed only in the leaves and flower buds, while only *BoTLP5* expressed in all organs tested. *BoTLP6* and *11* expressed only in flower buds,

indicating their probable role as allergen genes. Obviously, all genes described here showed higher level of expressions in leaves and flower buds compared to roots and stems. TLPs are now recognized as a new class of panallergens in food as well as in pollen (Breiteneder 2004). Moreover, the organs tested in this study share characteristics that make them particularly prone to pathogen attack. Adequate protection of these organs against pathogen attack is critical, and the production of an antifungal molecule could be part of a local defense strategy (Hamel and Bellemare 1995).

Fig. 1. Phylogenetic tree showing the relatedness of the deduced full-length amino acid sequences of 12 *Brassica oleracea* thaumatin-like proteins (BoTLPs) and 41 PR thaumatin-like proteins of other plant species. The phylogenetic tree was generated using MEGA5.05 program. The species names and the GenBank protein accessions numbers are indicated in brackets. *Brassica oleracea* (AAO12209), *Brassica rapa* (AAB95118), *Brassica rapa* subsp. *chinensis* (ACU65941 and BAJ16221), *Oryza sativa* Japonica Group (BAD30547 and BAC82958), *Arabidopsis thaliana* (AEE29691, AEE29690, NP001031064, AEE86963, AEE86962, AEE29829, NP173365, AED93336 and AEE29926), *Solanum lycopersicum* (CAB86199), *Nicotiana tabacum* (AAW66482, CAA33292 and BAA74546), *Zea mays* (NP001150507 and NP001151120) and BrTLP1 to 20 were taken from our previous study (Ahmed et al. 2013). The scale represents the frequency of amino acid substitution between sequences as determined by poisson evolutionary distance method.

Expression analysis after *P. carotovorum* subsp. *carotovorum* infection

Because EST analysis and previous studies indicated that thaumatin-like genes are biotic stress-related genes, we investigated the responses of the 12 *BoTLPs* identified in this study to infection by soft rot disease caused by necrotrophic bacteria, *P. carotovorum* subsp. *carotovorum*, which is a serious pathogen for *Brassica*. In the infected plants, expression of *BoTLP5*, *BoTLP8* and *BoTLP12* was differentially changed compared to mock-infected plants (Fig. 4A, B and C). *BoTLP5* and *BoTLP8* showed increased

level of transcripts after 48 h of infection but decreased after 72 h (Fig. 4A and B). Interestingly, the expression of *BoTLP5* and *BoTLP8* in mock-infected plants was higher at the earlier periods of treatment than the later. Accordingly, the expression was 19-fold higher for *BoTLP5* and 5-fold higher for *BoTLP8*, in the Pcc-infected plants than in mock-infected plants at 48 h. On the other hand, transcript level of *BoTLP12* was abruptly decreased just after infection and became almost inactive (Fig. 4C). It seems that only *BoTLP5*, 8 and 12 were induced among the *BoTLPs* after *P. carotovorum* subsp. *carotovorum* infection

Fig. 2. Alignment of deduced amino acid sequences of (A) Group I thaumatin-like proteins; (B) Group II thaumatin-like proteins and sweet-tasting thaumatin protein of *Thaumatooccus daniellii* (Bennett) Benth. (P02884) in each group using PIR. Asterisk marks show all 16 conserved cysteine residues, square bullets indicate REDDD motif in the highly conserved region (rectangle) of the domain and circular bullets indicate FF hydrophobic motif described by Petre et al. (2011) in identical positions among the aligned proteins. Numbers on the right margin indicate the positions of amino acid residues. The identical amino acids are marked in dark background, and more than 50% similarities in light.

Fig. 3. RT-PCR expression analysis of 12 *BoTLP* genes over different organs at *Brassica oleracea* plants. Lanes 1-4, PCR products of roots (R), stems (S), leaves (L) and flower buds (Fb)

and possibly other genes might be responsive to other biotic stress agents. Thus, the results suggest that *BoTLPs* are PR TLPs and have probable involvement with biotic stress resistance.

Previous molecular studies of TLP expression, localization and activity support a role for TLPs in host defense during pathogen infection. TLP up-regulation has been described in many higher plants infected by pathogens such as bacteria, oomycetes and fungi (Liu et al. 2010; Mukherjee et al. 2010). It has also been suggested that the expression of PR-5 proteins (TLPs) contributes to resistance to different fungi (Vleeshouwers et al. 2000; Monot et al. 2002; Suo and Leung 2002). A PR5 thaumatin-like protein from *A. thaliana* ATLP3 expressed in bacteria showed considerable antifungal activity against pathogenic fungi such as *V. alboatrum*, *V. dahlia*, *F. oxysporum* and *A.*

Fig. 4. Real-time quantitative PCR expression analysis of (A) *BoTLP5*, (B) *BoTLP8* and (C) *BoTLP12* genes in *Brassica oleracea* after infection with *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc). The error bars represent the standard error of the means of three independent replicates.

solani, as well as non-pathogenic fungi including *T. reesei* and *C. albicans* (Hu and Reddy 1997). Overexpression of cotton (*Gossypium barbadense* L.) thaumatin-like protein gene (*GbTLP1*) in tobacco exhibited considerable resistance against *V. dahliae* and *F. oxysporum* (Munis et al. 2010). Transgenic tobacco plants with thaumatin gene exhibited enhanced resistance with delayed disease symptoms against fungal diseases caused by *Pythium aphanidermatum* and *Rhizoctonia solani* (Rajam et al. 2007). A barley thaumatin-like protein (*Hv-TLP8*) in transgenic oilseed rape (*B. napus*) exhibited enhanced resistance to the clubroot pathogen (*Plasmodiophora brassicae*) (Reiss et al. 2009). Taken together, the above discussion suggests the probable association of these genes with biotic stress resistance.

In this study *BoTLP5*, 8 and 12 responded positively after *P. carotovorum* subsp. *carotovorum* infection and for that it is mentionable that these genes could be considered as useful resources for biotic stress resistance. Thus, *BoTLP5*, 8 and 12 might be promising candidate for engineering *Brassica* resistant to biotic stresses. The remaining genes also might be useful for other biotic stresses resistance in *Brassica*, thus, investigations involving screening of these genes with other pathogens is recommended.

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