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Expression, molecular characterization and detection of lipoxygenase activity of *tomloxD* from tomato

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**Abbreviations:** LOX, Lipoxygenases; JA, jasmonic acid; ABA, abscisic acid; RT-PCR, reverse transcriptase polymerase chain reaction; BMGY, buffered glycerol complex medium; MM, modified medium; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; cTP, chloroplast transit peptides; SMART, simple modular architecture research tool; BLAST, basic local alignment search tool; BPH, brown planthopper; SSB, stripped stem bore; LF, leaf folder.

**Key words:** Eukaryotic expression, lipoxygenase (LOX) activity, *Lycopersicon esculentum* Mill., mechanical injury, semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR), *TomloxD*, Western blot.

**INTRODUCTION**

Lipoxygenases (LOX) (linoleate: oxygen oxidoreductase, EC 1.13.11.12; LOXs) are a family of enzymes found ubiquitously in plants and mammals, but have also been detected in coral, moss algae, fungi, yeast and a number of bacteria (Kühn et al., 2005; Lang and Feussner, 2007; Liavonchanka and Feussner, 2006). LOXs catalyze the oxygenation of polyunsaturated fatty acids (Brash, 1999). Plant LOXs are ubiquitous and encoded by multigene families. LOXs can be found in all organs in plant (Loiseau et al., 2001). LOXs are almost involved in various physiological processes (Kolomiets et al., 2000; Veronesi et al., 1996).

Plant LOXs are a class of non-heme, iron-containing, monomeric proteins of about 95 to 100 kDa that is made of two domains. The amino-terminal domain of about 25 to 30 kDa is a β-barrel domain (domain I) (Andreou and Feussner, 2009; Corbin et al., 2007). Its exact function is yet unknown, but an involvement in membrane or substrate binding has been discussed (Andreou and Feussner, 2009; May et al., 2000; Tatulian et al., 1998). The carboxyl-terminal domain of about 55 to 65 kDa consists primarily of α-helices (domain II) and harbors the catalytic site of the enzyme (Andreou and Feussner,
LOX enzymes contain one iron atom per protein molecule. The iron-containing active site is in the center of domain II. The iron active site metal is a non-heme iron that is octaedrally coordinated by 5 amino acid side chains and a water or hydroxide ligand. In case of plant LOXs, these residues are always three histidines, one asparagine and the carboxyl group of the carboxyl-terminal conserved isoleucine (Androu and Feussner, 2009). The N-terminal domain makes only a loose contact with the C-terminal domain; this may be dispensable for plant lipoxygenases, because all the amino acid side chains responsible for catalysis are located in the C-terminal domain (Boyington et al., 1993; Minor et al., 1996).

LOX catalyzes the insertion of molecular oxygen into polyunsaturated fatty acids (PUFAs) that contain a (Z,Z)-1,4-pentadiene system to produce an unsaturated fatty acid hydroperoxide. Oxygen can be added to either end of the pentadiene system (regiospecificity). In higher plants, the natural substrates for these enzymes are linolenic (18:2) and linoleic (18:3) acids (Siedow, 1991). LOX-catalyzed incorporation of molecular oxygen into these fatty acids can occur either at carbon positions 9 or 13 (Brash, 1999), which leads to two possible products, the 9- and 13-hydroperoxy fatty acids (Siedow, 1991). In in vitro, most LOX prefer free fatty acids, though it has been shown that sterified fatty acids are also substrates for LOX in vivo (Feussner et al., 2001) suggesting that, membrane lipids could be substrates for oxylipin biosynthesis (Porta and Rocha-Sosa, 2002).

LOXs are responsible for the dioxygenation of polyenoic fatty acid in the production of fatty acid hydroperoxides, which are further metabolized to various lipid mediators. In plants, LOX can be further metabolized to yield volatile aldehydes and jasmonates (Feussner and Wasternack, 2002; Mosblech et al., 2009). These compounds have physiological functions in a variety of plant processes such as seed development, germination, vegetative growth, generation of fatty acid-derived flavor compounds, wounding, stress responses, senescence and cell signaling (Chen et al., 2004; Padilla et al., 2009; Porta and Rocha-Sosa, 2002; Siedow, 1991). Oxylipins produced by the 9-Lipoxygenase pathway in Arabidopsis regulate lateral root development and defense responses through a specific signaling cascade (Vellosoillo et al., 2007).

In plants, multiple LOX isoforms which exist are characterized by their different temporal and spatial distributions during plant development (Tamás et al., 2009). LOX genes isolated from different plant species show differential organ specific expression (Griffiths et al., 1999; Kolomiets et al., 2001). In addition, LOX protein and activity levels is regulated by different effectors such as the source/sink status, jasmonic acid (JA), abscisic acid (ABA) and by different forms of stress, such as wounding, insect attack, water deficiency, pathogen attack, or their elicitors (Bohland et al., 1997; Creeelman and Mullet, 1997; Gao et al., 2008; Hwang and Hwang, 2010; Jardim et al., 2010; Melan et al., 1993; Porta et al., 2008; Veronesi et al., 1996; Veronico et al., 2006; Wang et al., 2008; Zhou et al., 2009).

Products of the LOX in plants pathway have several diverse functions. LOX has been associated with some processes in a number of developmental stages (Kolomiets et al., 2001; Siedow, 1991). LOX have implicated some of the physiological processes which include wounding, pathogen attack, seed germination, fruit ripening, plant senescence, cell death and synthesis of ABA and JA (Gao et al., 2009; Hayashi et al., 2008; Hwang and Hwang, 2010; Marmey et al., 2007; Porta and Rocha-Sosa, 2002). Seed LOXs may function as storage proteins (Siedow, 1991). LOXs participate in the mobilization of storage lipids during germination (Feussner et al., 2001). During normal vegetative and reproductive growth, LOX is also used as storage for protein, participate in transference of lipid and response to nutrient stress and source/sink relationships (Fischer et al., 1999).

There are at least five lipoxygenases present in tomato (Lycopersicon esculentum Mill.) (Chen et al., 2004). Among the five tomato LOX genes, TomloxA, TomloxB, and TomloxE have high homology and are 72 to 77% identical with each other at the amino acid level, while TomloxC and TomloxD show 46% identity to each other and 42 and 47% identity to the TomloxA protein, respectively. TomloxB and TomloxC expression is enhanced by the ripening hormone ethylene, whereas TomloxA expression declines (Griffiths et al., 1999). TomloxC is expressed during ripening that can use both linoleic and linolenic acids as substrates to generate volatile C6 flavor compounds (Chen et al., 2004). The individual LOX isoforms are differentially regulated and may have distinct functions during growth and development of tomato plants.

In this study, the expression characters of TomloxD gene was analyzed and three-dimensional structure of TomloxD protein was predicted and given its characteristics. To determine the functional activity of the TomloxD, yeast cells synthesizing this protein were obtained. The enzyme activity analysis of TomloxD protein suggested that, TomloxD gene code is a lipoxygenase which participate in response to wounding.

MATERIALS AND METHODS

Growth of plants and treatments

All experiments were performed using a near-isogenic line of diploid tomato (L. esculentum Mill. cv Alisa Craig) plants. Tomato plants were grown in pot methods and maintained for 15 h of light (30 µE m⁻² s⁻¹) at 26°C and 9 h of dark at 19°C. The leaves were wounded by crushing the leaf with a hemostat, but the main vein of each leaflet cannot be wounded. The wounded leaves were incubated on the wet filter paper in closed can. The wounded leaves of seedlings
Table 1. Nucleotide sequences of primers used for PCR amplification in the present study.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Primer</th>
<th>Sequence (5' and 3')</th>
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<tbody>
<tr>
<td>1</td>
<td>TomD f</td>
<td>5'-ATTATTAGCCTTGCTAAAGAATT-3'</td>
</tr>
<tr>
<td>2</td>
<td>TomD r</td>
<td>5'-AATTAGATATCGATACTATTTGGAACA-3'</td>
</tr>
<tr>
<td>3</td>
<td>TomDS f</td>
<td>5'-GACAAAGCATTAGCAGTAAGTG-3'</td>
</tr>
<tr>
<td>4</td>
<td>TomDS r</td>
<td>5'-TAAATTGCGCCACACATCACAGC-3'</td>
</tr>
<tr>
<td>5</td>
<td>Actin f</td>
<td>5'-GTGAAATTGTGAGTGGAATTAGG-3'</td>
</tr>
<tr>
<td>6</td>
<td>Actin r</td>
<td>5'-TGAAGGGAAGCCAAAGATAGGC-3'</td>
</tr>
<tr>
<td>7</td>
<td>TomDJ f</td>
<td>5'-ACCAGGAATTTCCATCATACCATCAGTATCGATGATAAGATGAGCATGTTTGCTAAGAAATT-3'</td>
</tr>
<tr>
<td>8</td>
<td>TomDJ r</td>
<td>5'-GTCGCGCAGGCTCTATCATGACACTATTGGAA-3'</td>
</tr>
</tbody>
</table>

were collected after 0, 2, 6, 12 and 24 h treatment, respectively, frozen in liquid nitrogen and then stored at -80°C. The roots, flowers, younger and mature leaves, and fruits at the mature green, breaker and breaker + 4 days (B + 4) stages were collected and stored at -80°C, respectively.

Cloning of TomloxD gene

Total RNA was extracted from the seedlings using the Trizol reagent (Gibco-BRL, USA). One microgram of total RNA from each treatment was used for reverse transcription using oligo(dT)18 as 3' primer. Based on the TomloxD cDNA sequence (GenBank accession no. U37840.1), a pair of primers, TomD f and TomD r, was designed (Table 1). The TomloxD gene sequence was amplified from cDNA by reverse transcriptase polymerase chain reaction (RT-PCR) using TaKaRa LA TaqTM polymerase and 30 cycles of 94°C for 30 s, 58°C for 45 s and 72°C for 170 s. This PCR product was cloned into pMD18-T vector (Invitrogen, USA) and sequenced.

Structure prediction and homology modeling of the OsGSTL2 protein

The nucleotide sequence and the putative amino acid sequence were analyzed using blastP (http://www.ncbi.nlm.nih.gov/), the SMART (http://smart.embl-heidelberg.de/) and ChloroP (http://www.cbs.dtu.dk/services/ChloroP/), respectively. The soybean (Glycine max) LOX protein (LOX3) (Protein Data Bank code No.: 1no3A) was used as templates for constructing a structure model of TomloxD. The three dimensional structure of TomloxD protein was modeled using SWISS-MODEL, set automatically to seek appropriate known protein crystal structures as templates based on sequence similarity (http://swissmodel.expasy.org/). The models were selected according to model evaluation score calculated by Procheck and Whatcheck (Arnold et al., 2006; Schwede et al., 2003).

Expression analysis of TomloxD gene in tomato

In order to analyze expression of TomloxD gene in tomato, TomDS f and TomDS r were employed to produce a 290 bp PCR product (Table 1). The housekeeping gene, Actin gene from tomato was used as a control (GenBank accession no. U60480). The primers Actin f and r were designed to obtain a 201 bp amplification product (Table 1). The gene fragments of TomloxD and Actin were amplified using RT-PCR reaction. Amplification was carried out by initial denaturation at 94°C for 2 min followed by 28 cycles of 94°C denaturation for 30 s, 58°C annealing for 30 s and 72°C elongation for 30 s. PCR products from each amplification reaction were separated in a 2% (W/V) agarose gel. The relative expression level of TomloxD in different tissues was calibrated against the expression level of the inner control gene Actin.

LOX activity assays in wounded tomato leaves

Extraction of tomato lipoygenases were carried out according to the methods of Ohta et al. (1996) for purifying rice lipoygenases with several modifications. Tomato leaves (0.2 g) were homogenized with 1 ml of 50 mm sodium phosphate and 1.5% (w/v) Triton X-100 (pH 6.8) using a Polytron homogenizer and centrifuged at 18,000 g for 15 min. The supernatant was used as a crude extract and assayed for LOX activity.

LOX activity was measured spectrophotometrically at 234 nm by the methods of Shi et al. (1996). The reaction mixture (3.0 ml) contained 2.5 mM linoleic acid and 0.1% (w/v) Tween 20 in 0.2 M sodium Borate buffer (pH 9.0). Assays were carried out at 25°C for 15 to 30 s and absorbance at 234 nm derived from the conjugated diene chromophore of fatty acid hydroperoxides was monitored with a spectrophotometer (UV-160A, Shimadzu, Kyoto, Japan). One unit of activity was defined as the quantity of enzyme catalyzing an increase in absorbance of 0.01 at 234 nm/min under assay condition. The specific activities of the enzyme fractions were calculated based on the amount of protein in the fraction. Protein was estimated by the Bradford (1976) method using bovine plasma gamma globulin as a standard.

TomloxD eukaryotic expression

Construction of TomloxD eukaryotic expression vector

In order to express the TomloxD protein in Pichia pastoris expression host strain GS115, the reading frame sequence of the TomloxD gene was amplified with the combinations of specific primers TomDJ f and TomDJ r as detailed in Table 1. The forward primer TomDJ f was introduced to an EcoRI site and a His-Tag at the 5'-end and the reverse primer TomDJ r was introduced to a NotI site. The amplification product was cloned into pPIC9K vector (Invitrogen, Carlsbad, USA) by EcoRI and NotI. The recombinant plasmid was transformed into E. coli top 10 and was identified by restriction enzyme analysis and sequencing (Invitrogen in Shanghai).

Expression of TomloxD in Pichia pastoris GS115

The correct expression vector were transformed into P. pastoris strain GS115 and identified by PCR. Expression of TomloxD in P.pastoris GS115 was carried out according to the methods of Hu et al. (2009) with several modifications. Positive transformants were
grown overnight in 5 ml of buffered glycerol complex medium (BMGY) at 30°C with vigorous shaking. Cell cultures were harvested by centrifugation at 4,000 rpm for 10 min, resuspended in 50 ml of modified medium (MM) and grown for about 3 days at 30°C with vigorous shaking. The expression of TomloxD recombinant fusion protein was induced through 1.0% (v/v) methanol. The supernatant was obtained by centrifugation at 4000 rpm for 10 min at 4°C. Then the supernatant was concentrated by 30-fold ultrafiltration centrifugation. The concentrated supernatant was used to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), western blot analysis and enzyme assay. Protein concentration in crude extracts was determined with the BioRad Protein Assay Kit (Bradford, 1976).

Western blot analysis

The concentrated supernatant was separated in SDS-PAGE and transferred onto Immobilon membrane (Millipore, USA) with a semi-dry transfer cell (Millipore, USA). The recombinant TomloxD polypeptides expressed in P. pastoris strain GS115 was detected with an antibody raised against His-tag (Novagen, USA).

LOX activity assay of TomloxD

Protein concentration in supernatant was adjusted to 0.5 mg/ml. LOX activity assay of TomloxD was measured spectrophotometrically (Shi et al., 1996). One unit of activity was defined as the quantity of enzyme catalyzing an increase in absorbance of 0.01 at 234 nm/min under assay condition.

RESULTS

Structure prediction and homology modeling of the TomloxD protein

The ChloroP server predicted that, TomloxD has chloroplast transit peptides (cTP), which was a stretch of 77 amino acids in the N-terminal region of protein sequences. Both protein Basic Local Alignment Search Tool (BLAST) and the Simple Modular Architecture Research Tool (SMART) analysis showed that the TomloxD belonged to plant lipoxygenase and had two conserved domain. PLAT/LH2 domain spans over 140 residues (in position 74 and 213 of polypeptide), which is predicted to be a β-barrel structure. pfam: lipoxygenase spans over 671 residues (in position 222 and 892 of polypeptide), which is predicted to be the catalytic site in the C-terminal domain (residues 222 and 892).

The three-dimensional structure of TomloxD was modeled, based on the X-ray structure of the soybean LOX3 protein (Protein Data Bank code No.: Ino3A). The modeled residue range of TomloxD was approximately 820 amino acids (positions 88 and 908). Figure 1A shows the crystal structure of soybean LOX3 protein. The structure modeled by a protein structure modeling program modeller is displayed in Figure 1B. This structure was further checked by Procheck and Whatcheck. The three-dimensional structure alignments of TomloxD protein and soybean LOX3 protein is shown in Figure 1C, which suggested that these two proteins was high overlap. TomloxD contained all the functional domains typical of LOXs (Figure 1): a β-barrel structure in N-terminal region (residues 74 to 213) and a catalytic site in the C-terminal domain (residues 222 to 892). The 9 β-strands make the β-barrel structure whose residues are in positions 109 to 116, 129 to 133, 135 to 138, 146 to 152, 164 to 170, 174 to 182, 190 to 193, 196 to 199 and 208 to 211, respectively (Figure 1B). The catalytic site consists of a αβαααββααααααααααααααα structural motif, which contains 14 α-helices and 6 β-strands. The residues of α-helices
Figure 2. The expression analysis of \textit{TomloxD} by semi-quantitative RT-PCR. A) The expression of \textit{TomloxD} in tomato tissues. Lane 1 to 4, roots, flowers, younger and mature leaves, respectively; Lane 5 to 7, fruits at the mature green, breaker and breaker+4 days stages, respectively. B) The relative expression of \textit{TomloxD} in mechanical injury leaves. Lane 1 to 5 is the treatment time of 0, 2, 6, 12 and 24 h, respectively.

are in position 223 to 238, 320 to 348, 354 to 363, 373 to 379, 414 to 422, 481 to 494, 542 to 581, 591 to 615, 629 to 644, 678 to 702, 705 to 731, 741 to 770, 810 to 824 and 846 to 873, respectively, and the residues of 6 β-strands are in position 252 to 259, 427 to 430, 475 to 480, 501 to 511, 512 to 525 and 530 to 536, respectively (Figure 1B).

Expression characters of \textit{TomloxD} in tomato

Expression of \textit{TomloxD} in tomato tissues

The \textit{TomloxD} mRNA was detected in the roots, flowers, younger and mature leaves and fruits at the mature green, breaker and breaker+4 days stages from tomato plants using semi-quantitative RT-PCR analysis (Figure 2A). The \textit{TomloxD} transcript was higher in roots, flowers, younger leaves and fruits of breaker+4 days stage, whereas lower in mature leaves, fruits from the mature green and breaker stages.

Expression of \textit{TomloxD} in response to mechanical injury

Semi-quantitative RT-PCR analysis demonstrated that, \textit{TomloxD} transcript was up regulated and reached the peak around 2 h, followed by a decrease at 6 h and a further decrease at 12 h after injury (Figure 2B). LOX activity of crude extracts from the tomato leaves was measured with linoleic acid as the substrate. Each point reported is the average of three plants and each treatment was analyzed in three independent experiments. The change of LOX activities exhibited the same as that of \textit{TomloxD} mRNA in wounded leaves. The LOX activity showed a peak level within 2 h, which was about 1.84 times more than that of the untreated leaves, but also had a decline thereafter (Figure 3). The findings showed \textit{TomloxD} expression responses to mechanical injury.

Expression of \textit{TomloxD} transgene in yeast

To investigate the function of \textit{TomloxD} protein, the \textit{TomloxD} gene was cloned into pPIC9K vector (Invitrogen, USA) and was transformed into \textit{P. pastoris} expression host strain, GS115. The supernatant was concentrated by ultrafiltration centrifugation. The concentrated supernatant was analyzed by SDS–PAGE (Figure 4). A novel protein band of about a 100 kDa molecular weight was obtained in \textit{TomloxD} transgenic yeast.

As the target protein contains a 9×His-Tag recognition sequence in its N terminal, we analyzed the recombinant protein by Western blotting with anti-his antibody. A single target protein band was detectable in the expression supernatant after ultrafiltration centrifugation, which showed
that the contained TomloxD and His-Tag fusion protein was expressed in TomloxD transgenic yeast. But under the same culture conditions, no exogenous TomloxD was detected in pPIC9K vector transformed yeast (Figure 5). The target protein band was weaker in Western-blot analysis than SDS–PAGE. That is because TomloxD protein is a large molecule with a predicted molecular weighs of 102.31 kilodaltons, it is very difficult to transfer the target protein onto Immobilon membrane.

**LOX activity analysis of TomloxD**

LOX activity of supernatant from the TomloxD transgenic yeast was measured with linoleic acid as the substrate. The findings showed that, TomloxD transgenic yeast induced with 1.0% (v/v) methanol contains high levels of LOX activities and pPIC9K transformed yeast has hardly LOX activity (Figure 6).

**DISCUSSION**

TomloxD has a chloroplast transit peptide, which consists of 77 amino acids in the N-terminal region. The three dimensional structure alignments of TomloxD protein and soybean LOX3 protein suggested that, TomloxD contained all the functional domains typical of LOXs. Western blot analysis showed that TomloxD protein was synthesized in transformed yeast cells. The enzyme activity analysis of TomloxD protein suggested that TomloxD gene code has a lipoxygenase.

Multiple isoforms of LOX have been detected in a wide range of organisms (Chen et al., 2004). Lipogenase activity in plants has been observed in several cell fractions, including chloroplasts, mitochondria, vacuoles, lipid bodies and membranes (Liavonchanka and Feussner 2006; Tamás et al., 2009). According to the position of addition of the -OOH moiety in the primary hydroperoxide product using linoleic or linolenic acids as substrates, LOX enzymes can be grouped into two types: 9-LOX, which specifically forms 9-hydroperoxides (9-HPOs) and 13-LOX, which specifically forms 13-hydroperoxides (13-HPOs). The product of the reaction of linoleic acid with soybean cotyledon lipoxygenase-1 is almost exclusively 13-hydroperoxy linoleic acid (Axelrod et al., 1981). In potato (Solanum tuberosum), Lox1 is expressed in tubers and roots and uses linoleic acid to produce predominantly 9-HPOs. The amino acid sequences of TomloxA, TomloxE, and TomloxB have strong homology to that of potato Lox1 and show 92, 75, and 69% identity, respectively. Taking into account that the majority of the hydroperoxides formed by LOX activity in tomato are the 9-isomers (Galliard and Matthew, 1977; Smith et al., 1997) and that TomloxB, TomloxE, and TomloxA have high expression levels in fruit, it is most likely that, TomloxA, TomloxB, and TomloxE may produce 9-HPOs (Chen et al., 2004). TomloxC is a chloroplast-targeted lipoxygenase isoform that can use both linoleic and linolenic acids as substrates and produce 13-hydroperoxy products. The 13-HPOs formed in tomato appear to be
metabolized further by the action of the 13-hydroperoxide lyases (13-HPLs) to give rise to hexanal and the corresponding alcohols (Chen et al., 2004). The *TomloxD* protein also has a chloroplast target signal. Sequence comparison showed that the *TomloxD* protein sequence has 92% identity at the amino acid level with that of potato Lox3, which is mostly expressed in potato leaves and roots and produces almost exclusively 13-HPOs (Royo et al., 1996). It is most likely that *TomloxD* may produce 13-HPOs.

*TomloxD* may be capable of generating 13-HPOs, but *TomloxD* does not play a key role in the generation of flavor volatiles in fruit (Chen et al., 2004). 13-HPOs can be used as substrates by several different enzymes (Schaller, 2001). The metabolic fate of 13-hydroperoxides may depend on their colocalization or association with specific downstream enzymes.

LOXs are also involved in JA synthesis, in response to mechanical injury and in defense responses against insect pests and microbial pathogens. For example, in transgenic *Arabidopsis* plants, suppression of the chloroplast targeted LOX2 gene resulted in the absence of wound inducible JA accumulation and reduced expression of the wound and JA-inducible *vsp* gene (Bell et al., 1995). Potato plants with silenced LOX H3, a *TomloxD* homolog, showed greatly reduced proteinase inhibitor expression, indicating a role in defense signaling (Royo et al., 1999). Inactivation of the lipoxygenase ZmLOX3 increases susceptibility of maize to *Aspergillus* spp. (Gao et al., 2009). OSHI-LOX is a chloroplast-localized type 2 13-lipoxygenase gene of rice, whose transcripts were up-regulated in response to feeding by the rice striped stem borer (SSB) *Chilo suppressalis* and the rice brown plant hopper (BPH) *Niparvata lugens*, as well as by mechanical wounding and treatment with JA. Antisense expression of OSH-LOX (as-lox) reduced striped stem bore (SSB) or BPH-induced JA and trypsin protease inhibitor (TrypPI) levels, increased the damage caused by SSB and LF (leaf folder) larvae (Zhou et al., 2009). OsLOX1 product is involved in tolerance of the rice plant to wounding and BPH attack (Wang et al., 2008). Semi-quantitative RT-PCR analysis demonstrated that the product of *TomloxD* is expressed principally in roots, flowers, younger leaves and fruits of breaker+4 days stage and at a very low level in mature leaves, fruit at the mature green stage and at onset of ripening (Figure 2A). The *TomloxD* transcript was upregulated by mechanical injury and the change of LOX activities exhibited the same as that of *TomloxD* transcript after mechanical injury (Figure 2B and 3). The findings suggested the *TomloxD* expression was response to mechanical injury. The results of this study are consistent with the previous work of Heitz et al. (1997), which concluded that *TomloxD* is mainly expressed in tomato leaf and is up-regulated in leaves in response to wounding (Heitz et al., 1997). Hence, *TomloxD* may play a role as a component of the octadecanoid defense-signaling pathway, as has been demonstrated for its corresponding homologous genes in potato and *Arabidopsis* plants (Bell et al., 1995; Royo et al., 1999).

In summary, the *TomloxD* protein is a chloroplast-targeted lipoxygenase isoform that contains all the functional domains typical of LOXs and has LOX activity. The *TomloxD* may use both linoleic and linolenic acids as substrates and produce 13-hydroperoxy products. The expression of *TomloxD* gene was induced by mechanical injury. The characteristics of the *TomloxD* gene and its product indicate that, it is a strong candidate and a component of the octadecanoid pathway and may play a role in the defense-signaling pathway in tomato plants. In order to elucidate the precise physiological functions of *TomloxD* gene, further biochemical and molecular experiments are in progress in our laboratory.

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Sims)


