Profiling expression of lipoxygenase in cucumber during compatible and incompatible plant-pathogen interactions

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Abstract We compared lipoxygenase (LOX) expression in cucumber in response to host and non-host pathogens. Our results displayed significant difference in expression of LOX between compatible and incompatible interaction at 12, 24 and 48 h after inoculation. Moreover, LOX expression at 72 h after inoculation was similar in both compatible and incompatible interaction. It seems that early induction of LOX plays a crucial role in plant defense against pathogens.

Keywords Lipoxygenase · Expression · Cucumber · Compatible interaction · Incompatible interaction

Plants are exposed to pathogens invasion constantly. During infection and colonization of the plant tissue, some defense genes are expressed to suppress the pathogen attack. Lipoxygenase (LOX; EC 1.13.11.12) are a group of non-heme iron-containing dioxygenases that initiate the degradation of free fatty acids and esterified lipids through different branches of the LOX pathway. LOX proteins catalyze the addition of oxygen to either end of a (Z, Z)-1,4-pentadiene system of polyunsaturated fatty acids to produce an unsaturated fatty acid hydroperoxide (Brash 1999). LOX genes have been reported in some plant species such as peanut, soybean, cucumber, barley, lentil, tomato, tobacco, rice, common bean, pea, potato and broad bean (Porta and Rocha-Sosa 2002). Expression of LOX is affected by various factors like

developmental stage (Kolomiets et al. 2001), Jasmonic acid (Creelman and Mullet 1997), abiotic stress (Porta et al. 1999), abscisic acid and pathogen invasion (Melan et al. 1993). The objective of this study was to compare expression of LOX in compatible and incompatible cucumber-pathogen interactions. *Pseudomonas syringae* pv. *lachrymans*, the causal agent of angular leaf spot of cucumber and *P. syringae* pv. *syringae*, the etiological agent of bacterial canker of stone fruits, were used for investigation of compatible and incompatible cucumber-pathogen interactions, respectively.

Cucumber (*Cucumis sativus* cv. *salar*) seeds were surface-sterilized by 1.0 % sodium hypochlorite (20 % household bleach) for 10 min, then sown in quartz sand in 12 cm plastic pots in a growth chamber. Plants were grown with a 16 h photoperiod at 28 °C. *P. syringae* pv. *syringae* D2 (Rezaie 2013) and *P. syringae* pv. *lachrymans* V5 (Movahedi Parizi 2013) were used as a non-host and host pathogens, respectively. The inoculum was prepared in sterile distilled water at a concentration of about 1×10^7 cfu/ml. Moreover, sterile water was used as negative control. Total RNA was extracted from the leaves using a RNA isolation kit (DENAzist, Iran) according to the manufacturer's guidelines. The RNA pellet was washed with ice-cold 75 % ethanol, air-dried and dissolved in 30 µl of DEPC-water. Isolated RNA was treated with DNase I (Fermentas) according to the manufacturer's instruction to remove genomic DNA contamination. Total RNA concentrations were measured at 260 and 280 nm by NanoDrop™ 1000 Spectrophotometer (USA) and the purity of the total RNA extracted was determined as the ratio of optical density at 260 nm to that at 280 nm (OD260/OD280) with values between 1.8 and 2.1. Finally, cDNA synthesis was performed on total RNA using cDNA synthesis kit (Thermo Scientific, China), according to manufacturer's instructions.

RT-PCR experiments were performed using RealQ PCR 2× Master Mix (Ampliqon, Denmark). Each reaction was made in

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Fig 1 Expression analysis of LOX by RT-PCR during infection of cucumber by *P. syringae* pv. *syringae* (incompatible interaction) and *P. syringae* pv. *lachrymans* (compatible interaction). Quantification of gene expression was carried out using the comparative Ct method (Livak and Schmittgen 2001). Relative expression of the gene is reported as the number of fold increase of the transcript level in infection experiments relative to each corresponding control sample

triplicate. Primers 5' TCAAGTGCCCCCATGGACTTAG 3' and 5' TCATGTTTCTTGTCAGCGTGGCC 3' (Zhao et al. 2010) were used to detect the otherness of LOX gene relative expression to pathogen attack. Additionally, 18S ribosomal RNA (18S rRNA) gene of *Cucumis sativus* with corresponding primers NS1 5' GTAGTCA-TATGCTTGTCTC 3' and NS2 5' GGCTGCTGGCACCAGACTTGC 3' (Cantarello et al. 2005) was used as a housekeeping gene. Total reaction volume was 25 μ l and included 10 μ l (2X) SYBR green mastermix, 100 ng of cDNA, 0.5 μ l of 10 μ M of each forward and reverse primers and volume adjusted with water. Thermal cycling conditions consisted of 95 $^{\circ}$ C for 3 min, 40 cycles of 95 $^{\circ}$ C for 1 min, 60 $^{\circ}$ C for 1 min, and 72 $^{\circ}$ C for 1 min, and final extension at 72 $^{\circ}$ C for 5 min. The real-time PCR was performed in a Bioneer (China). The experiments were repeated four times for each sample. The CT values of target and house-keeping genes were used for analysis of data. Relative gene expression was calculated using the comparative $\Delta\Delta C_T$ method according to Livak and schmittgen (2001). Comparisons among treatments were performed SAS 9.1 (SAS Institute, Cary, NC, USA) and a probability of $P < 0.05$ was considered significant.

Expression of LOX in incompatible interaction increased 2.1-, 6.1-, 8.1- and 6.4-fold at 12, 28, 48 and 72 h after inoculation compared to control, respectively. On the other hand, LOX expression in compatible interaction increased 1.1-, 1.4-, 4.2- and 6.3-fold at 12, 24, 48 and 72 h after inoculation compared to control, respectively. Significant difference was observed in expression of LOX between compatible and incompatible interaction at 12, 24 and 48 h after inoculation, while no significant difference was detected at 72 h after inoculation. The highest expression was recorded at 72 and 48 h after inoculation in compatible and incompatible interaction, respectively (Fig 1). Our results showed that LOX has an important role in cucumber defense against pathogens that in

agreement with previous studies. LOX expression increases in inoculated tomatoes with *Pseudomonas putida* (Mariutto et al. 2011). In *Arabidopsis thaliana*, the expression of LOX is stimulated by pathogens, abscisic acid, and methyl jasmonate (Melan et al. 1993). In tobacco, LOX expression enhances upon infection by *Phytophthora parasitica* var. *nicotianae*. LOX expression appear earlier in an incompatible plant-pathogen interaction than in a compatible one, demonstrating the role of LOX in plant defense against pathogen infection (Rance et al. 1998). LOX expression in incompatible interaction of common bean-*Pseudomonas syringae* pv. *phaseolicola* is higher than compatible interaction (Croft et al. 1993). Some products of LOX metabolism are essential to induce hypersensitive response at the site of infection in an incompatible interaction that limits pathogen growth. It has been approved that LOX mediated lipid oxidation is essential in causing membrane damage during hypersensitive response (Rusterucci et al. 1999). Jalloul et al. (2002) displayed faster and more expression of LOX in incompatible interaction of *Xanthomonas*-cotton than compatible interaction. We demonstrated that early induction of LOX is crucial to suppress the pathogen. In incompatible interaction, the level of LOX was evaluated faster than compatible interaction. Such information can be considered in plant protection programs.

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