Bioinformatics Identification of the Lipoxygenase Gene Family and Analysis of Their Gene Expression Characteristics in *Physcomitrella Patens*

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Abstract [**Objectives**] To investigate the structure and function of the lipoxygenase (LOX) gene family in *Physcomitrella patens*. [**Methods**] This study employed bioinformatics methods to identify and predict *LOX* gene family members. Quantitative real-time PCR (qRT-PCR) was utilized to analyze the expression patterns of *LOX* genes at different stages of *Botrytis cinerea* infection. [**Results**] The *P. patens LOX* gene family comprises eight putative proteins, including two 12-LOX-type members and six 13-LOX-type members. Among the eight LOX proteins, PpLOX7 exhibited the lowest molecular weight and shortest amino acid sequence. PpLOX7 was identified as a basic protein with an isoelectric point (*pI*) of 8.54, while all other members were acidic. Subcellular localization analysis indicated that PpLOX7 was localized to the chloroplast, whereas the remaining members were distributed in the cytoplasm. Secondary structure prediction showed that all eight proteins were predominantly composed of random coils and α-helixes. Chromosomal mapping revealed that the *LOX* genes were distributed across 7 of the 27 chromosomes in *P. patens*, with *PpLOX1* and *PpLOX2* tandemly arranged on chromosome 15. The qRT-PCR analysis demonstrated distinct expression patterns among the eight *PpLOX* genes following *B. cinerea* infection. *PpLOX1-3* and *PpLOX7* were upregulated to varying degrees, suggesting their potential involvement in the early defense response of *P. patens* against *B. cinerea*. Notably, *PpLOX2* exhibited highly significant differential expression, making it a key candidate for further investigation. [**Conclusions**] This study provides foundational insights into the functional roles of the *LOX* gene family in *P. patens* during biotic stress responses.

Key words *Physcomitrella patens*, Lipoxygenase, Bioinformatics, Gene expression

0 Introduction

The moss *Physcomitrella patens* belongs to the genus *Physcomitrella* within the family Funariaceae and order Funariales. The genome sequencing of *P. patens* has been completed[1]. Due to its ease of cultivation, the dominance of the gametophyte in its life cycle, and the high efficiency of homologous recombination in its nuclear genome, it has become an ideal model system for plant molecular biology research. Lipoxygenase (EC 1.13.11.12; LOXs), also known as lipoxidase or fatty acid oxygenase, is widely present in both animals and plants. LOXs are key enzymes in the fatty acid oxidation pathway and are closely associated with plant growth and development[2], senescence[3], as well as stress tolerance[4]. Lipoxygenases often exist in the form of gene families[5]. It has been reported that there are six *LOX* gene family members in *Arabidopsis thaliana*[6] and fourteen in tomatoes[7]. *P. patens*, as a lower group among higher plants, has become an ideal plant material for studying gene functions, but there are few reports on the disease resistance function of lipoxygenases. In this study, bioinformatics was used to identify and predict the members of the *LOX* gene family in *P. patens*. The expression patterns of *LOX* gene family members during *Botrytis cinerea* infection were analyzed using qRT-PCR. This research aims to provide foundational information for the identification and functional analysis of the *LOX* gene family in *P. patens* and to offer clear candidate genes at the molecular level for studying the disease resistance function of LOX in this plant.

1 Materials and methods

1.1 Experimental materials The *P. patens* was obtained from Professor Yikun He at Capital Normal University, and the *B. cinerea* strain c023 was obtained from Professor Ling Xu at East China Normal University.

1.2 Gene identification and classification The HMMsearch method was performed to identify protein sequences containing the lipoxygenase (PF00305) gene family domain[8]. The search results were used to generate a new protein HMM file, which was manually screened using SMART (http://smart.embl-heidelberg.de/) to remove sequences lacking the lipoxygenase (PF00305) domain. The remaining sequences were aligned with genomic data from NCBI, resulting in the identification of eight candidate genes.

1.3 Multiple sequence alignment and phylogenetic analysis Sequences containing the conserved LOX domain were aligned using ClustalW (https://www.genome.jp/tools-bin/clustalw). A phylogenetic tree was constructed using the maximum likelihood (ML) method in MEGA 10 (https://www.megasoftware.net/).

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The Neighbor-Joining (NJ) method was employed with 1000 bootstrap replicates. LOXs protein sequences from tobacco, kiwifruit, rice, soybean, porphyra, and carrageen were retrieved from the NCBI database (https://www.ncbi.nlm.nih.gov/). The amino acid sequences of Arabidopsis thaliana were obtained from the TAIR database (https://www.arabidopsis.org/).

1.4 Protein physicochemical properties, conserved domain analysis, and gene structure ProtParam[9] (http://expasy.org/tools/protparam.html) was used to predict the isoelectric point (pI), amino acid length, and molecular weight (MW) of eight protein sequences. The amino acid sequences of the candidate genes from *P. patens* were submitted to MEME (v4.8.1)[10] (http://meme.nbcr.net/meme/cgi-bin/meme.cgi) to predict the conserved domains of PpLOXs proteins. The secondary structures of the LOX proteins were predicted using the HNN Secondary Structure Prediction method (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html).

PLOC (http://www.csbio.sjtu.edu.cn/bioinf/Cell-PLoc-2/) was employed with the Plant-mPLOC (plant subcellular localization) option to determine the subcellular localization information of the *P. patens* lipoxygenase gene family members. The E-values corresponding to the proteins of the *P. patens* lipoxygenase gene family were determined by screening the obtained protein files.

1.5 Chromosomal localization of *LOX* genes in *P. patens* Online website (http://mg2c.iask.in/mg2c_v2.0/) was used to analyze the chromosomal localization of LOX family member genes, and the localization map was refined using the visualization software TBtools.

1.6 Cis-acting elements A total of eight identified gene family members were selected, and the 2 000 bp upstream sequences were defined as promoter regions. Cis-acting elements were predicted using PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/).

1.7 Expression patterns of *LOXs* in *P. patens* The expression patterns of the *PpLOXs* gene family will be analyzed using the Physcomitrella eFP Browser (http://bar.utoronto.ca/efp_physcomitrella/cgi-bin/efpWeb.cgi)[11], available through the BAR platform (http://bar.utoronto.ca/)[12].

1.8 Expression analysis of *LOX* gene family members in *P. patens* under *B. cinerea* stress The expression patterns of *LOX* family genes at 0 – 3 d after inoculation with *B. cinerea* were analyzed using quantitative real-time PCR (qRT-PCR). Total RNA was extracted from the samples using the Plant RNA Kit (BioTeKe, Beijing, China). First-strand cDNA was synthesized with the PrimeScript™ RT Reagent Kit (TaKaRa, Dalian, China) and used as the template for qRT-PCR. Gene-specific primers were designed using Primer software, and the primer sequences (Table 1) were synthesized by Sangon Biotech (Shanghai, China). The *P. patens Actin*3 gene was used as the internal reference. qRT-PCR was performed on a Real-Time PCR System (ABI 7500, USA) using SYBR Green (TaKaRa, Dalian, China) as the fluorescent dye. The thermal cycling conditions were as follows: 94 ℃ for 30 sec; 40 cycles of 94 ℃ for 5 sec, 55 ℃ for 15 sec, and 72 ℃ for 10 sec.

Table1 Specific primers used for quantitative RT-PCR

Primer name	Sequences of forward primer (5'-3')	Sequences of reverse primer (5'-3')
qPpLOX1-F/R	ATTGAAGGTAGCTTGGAAGGCC	CAAGAAAAGAAGTGTTCGGGTG
qPpLOX2-F/R	TTCCAAGAAGTAATGAGGCCCGAC	CCTTCCAAGCTACCTTCAATATG
qPpLOX3-F/R	CATGTGAGAGAGTTGTACGTAC	GAGAGTCTCAATAAGCAAGGG
qPpLOX4-F/R	GACATTTCCACCCTACAAAGAC	CCTGGAAGAGACAACTCAATAC
qPpLOX5-F/R	CTATCCAAGCATTGTACTCCAG	TAGGGTACGTGAGGATGATT
qPpLOX6-F/R	CACTTTACTTAAGGAGTGGCC	GTAGGGAAGGAAGATATCATGG
qPpLOX7-F/R	CTCACATTTCCTTTACCTGGAG	CTTCTCGACTGTAAGATCCTC
qPpLOX8-F/R	GAGATGACGAGAGAGAGAAATC	CTCGAGACAGTTGATAACACAG
Actin 3	CGGAATGGTGAAGGTATGAT	CACGATGTGAAGAAGACGAT

1.9 Data analysis The relative expression level was calculated using the formula $2^{-\Delta CT}$[13]. The data were organized and represented in tabular form using Microsoft Office 2010.

2 Results and analysis

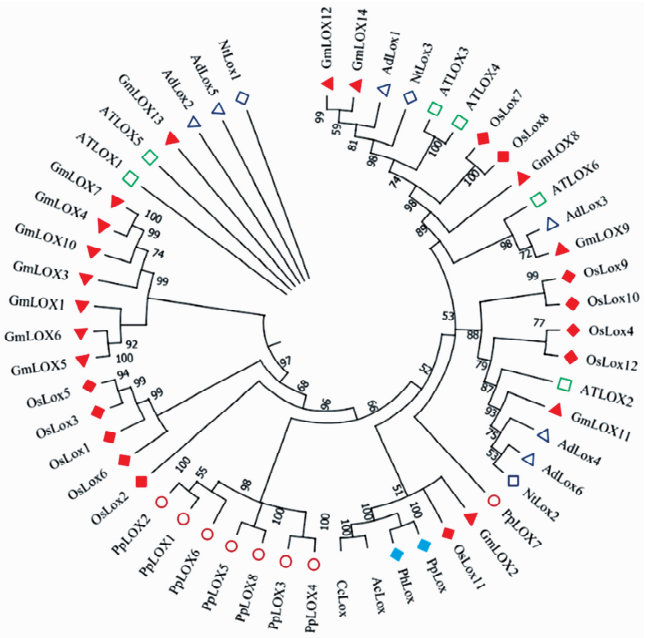
2.1 Identification and phylogenetic analysis of LOXs in *P. patens* In this study, members of the *LOX* gene family in *P. patens* were identified using Hidden Markov Model (HMM) and BLAST. A total of eight *LOX* family members were ultimately identified and sequentially numbered according to their accession numbers, designated as *LOX1* to *LOX8*. A phylogenetic tree was constructed using Clustal X and MEGA10 software. As shown in Fig. 1, the members of the *P. patens* lipoxygenase gene family exhibit high homology between *PpLOX1* and *PpLOX2*, as well as between *PpLOX5* and *PpLOX8*, and between *PpLOX3* and *PpLOX4*. In contrast, *PpLOX7* shows low similarity to other family members and is located on a distinct branch. Overall, the phylogenetic tree reveals that during the evolution from lower aquatic species such as *Pyropia* and *Chondrus*, which possess a single *LOX*, to bryophytes and dicot plants, the number of *LOXs* increased from few to many.

2.2 Analysis of physicochemical properties, conserved domains, and gene structure of *P. patens* LOX proteins Using the online tool ProtParam to predict the primary structure of proteins, the molecular weights of the eight members of the *P. patens*

lipoxygenase family were found to range between 71.5 and 108.5 kDa, with PpLOX7 being the lowest at only 71.5 kDa. The amino acid lengths ranged from 640 to 956 aa, with PpLOX7 being the shortest at 640 aa. The highest theoretical isoelectric point was observed for PpLOX7, with a pI value of 8.54, indicating it is a basic protein, while all other family members were acidic. Subcellular localization analysis revealed that, except for PpLOX7, which is localized in the chloroplast, all other members are distributed in the cytoplasm. Among the LOX family members in *P. patens*, the number of amino acids and molecular mass showed a positive correlation in all members except PpLOX6.

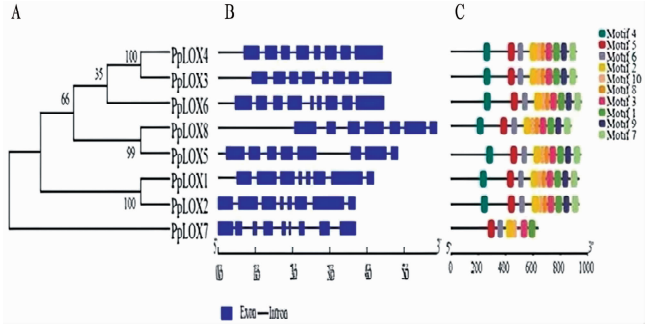
As shown in Table 3, the LOX proteins of *P. patens* contain 10 distinct protein domains, with widths ranging from 29 to 50 aa. Among them, motif8 and motif10 have the smallest width of 29 aa. PpLOX7 lacks the conserved domains motif4, motif7, and motif9. Analysis of the intron-exon structure of the LOX family members in *P. patens* (Fig. 2) reveals that most family members contain 7 introns, with a maximum of 8 introns. PpLOX8 has the fewest introns, containing only 6.

Analysis of HNN Secondary Structure Prediction revealed that the secondary structures of the eight proteins are predominantly composed of random coils and α-helices. Among these, random coils consistently accounted for the highest proportion, with PpLOX6 reaching up to 44.77%. In contrast, β-turns constituted the smallest proportion, with a minimum of approximately 5%. α-helices, extended strands, β-turns, and random coils are distributed throughout the entire protein structures (Table 4).



NOTE Scale shows evolutionary distance. Accession number of Arabidopsis LOX proteins: ATLOX1. AT1G55020.1; ATLOX2. AT3G45140.1; ATLOX3. AT1G17420.1; ATLOX4. AT1G72520.1; ATLOX5. AT3G22400.1; ATLOX6. AT1G67560.1. Accession number of *Actinidia chinensis* LOX proteins: AdLox1. DQ497792; AdLox2. DQ497797; AdLox3. DQ497795; AdLox4. DQ497793; AdLox5. DQ497796; AdLox6. DQ497794. Accession number of *Nicotiana tabacum* LOX proteins: NtLox1. AAP83134; NtLox2. AAP83137; NtLox3. 83138; Accession number of *Oryza sativa indica* LOX proteins: OsLox1. Q76I22; OsLox2. Q0DJB6; OsLox3. P29250; OsLox4. Q6H7Q6; OsLox5. Q7G794; OsLox6. Q53RB0; OsLox7. Q7XV13; OsLox8. Q8H016; OsLox9. P38419; OsLox10. Q84YK8; OsLox11. Q01S17; OsLox12. Q9FSE5. Accession number of soybean LOX proteins: GmLOX1. P08170; GmLOX2. P09435; GmLOX3. P09186; GmLOX4. P38417; GmLOX5. AAA96817; GmLOX6. P24095; GmLOX7. AAC49159.1; GmLOX8. XP_003541736.1; GmLOX9. KRH42646.1; GmLOX10. NP_001237338.2; GmLOX11. KPH29668.1; GmLOX12. KPH69027.1; GmLOX13. KRH68548.1; GmLOX14. KRH47615.1. Accession number of *Porphyra* LOX proteins: PhLox. AAA61791. Accession number of Carageen LOX proteins: CcLox. XP_005718273.1.

Fig. 1 Phylogenetic tree for the gene family of *LOX* in *Physcomitrella patens*, kiwi, *Arabidopsis thaliana* and tobacco



NOTE A. intron-exon structure, B. distribution of exons and introns in the Lipoxygenase family: blue for exons, black line for introns, C. motifs model of the protein domain of *LOX* gene family.

Fig. 2 Analysis of the intron-exon structure of the LOX family members in *Physcomitrella patens*

Table 2 Physical-chemical analysis and subcellular localization of predicted protein of the *LOX* gene family in *Physcomitrella patens*

Gene name	E-value	*pI*	Mw//KDa	Length of protein//aa	Subcellular localization
PpLOX1	7.8e-271	6.19	105.3	938	Cytoplasm
PpLOX2	1.0e-270	6.06	105.3	938	Cytoplasm
PpLOX3	1.2e-264	6.65	103.2	920	Cytoplasm
PpLOX4	5.6e-262	6.44	103.6	920	Cytoplasm
PpLOX5	1.7e-285	5.45	108.5	951	Cytoplasm
PpLOX6	3.6e-292	6.44	106.2	956	Cytoplasm
PpLOX7	1.5e-154	8.54	71.5	640	Chloroplast
PpLOX8	1.1e-273	6.88	100.0	880	Cytoplasm

Table 3 The motifs structure of *LOX* gene family in *Physcomitrella patens*

Motif name	Width aa	E-value	Logo
Motif1	50	4.5e-211	
Motif2	50	9.3e-183	
Motif3	50	4.4e-159	
Motif4	50	3.7e-134	
Motif5	48	1.6e-114	
Motif6	41	8.3e-108	
Motif7	50	8.9e-108	
Motif8	29	4.0e-072	
Motif9	44	5.9e-081	
M0tif10	29	6.3e-072	

Table 4 Secondary structure simulations of the *LOX* gene family proteins in *Physcomitrella patens*

Gene ID	Secondary structure			
	AlpHa helix	Extended strand	Beta turn	Random coil
PpLOX1	38.27	13.65	5.01	43.07
PpLOX2	38.70	13.75	4.69	42.86
PpLOX3	36.63	15.54	5.54	42.28
PpLOX4	37.61	14.57	5.98	41.85
PpLOX5	39.01	13.56	5.05	42.38
PpLOX6	36.72	14.02	4.50	44.77
PpLOX7	37.34	13.75	4.22	44.69
PpLOX8	37.05	14.55	4.54	42.95

Based on the genomic location information of each member of the lipoxygenase (LOX) gene family in *P. patens*, the eight *LOX* genes were mapped onto the chromosomes (Fig. 3). The eight genes are distributed across seven of the 27 chromosomes of *P. patens*: *PpLOX6* is located on chromosome 1, *PpLOX7* on chromosome 4, *PpLOX8* on chromosome 7, *PpLOX5* on chromosome 11, *PpLOX4* on chromosome 14, *PpLOX1* and *PpLOX2* are co-located on chromosome 15, and *PpLOX3* is located on chromosome 17. The distribution of the *LOX* gene family members across the chromosomes is dispersed. Specifically, the eight genes are spread over seven of the 27 chromosomes, with no evidence of clustered distribution on any single chromosome.

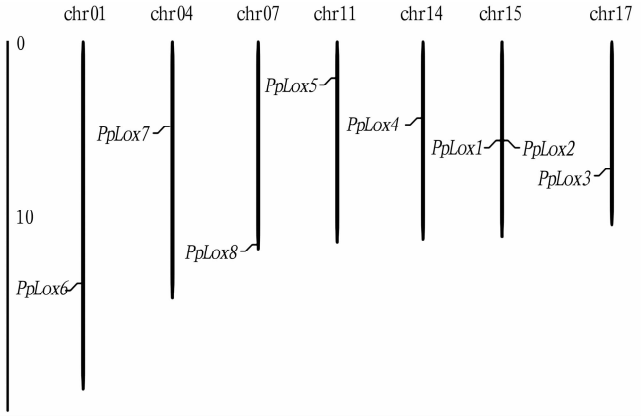


Fig. 3 Chromosomal locations analysis of the *LOX* gene family in *Physcomitrella patens*

2.3 Promoter analysis of the *PpLOXs* gene family Analysis of the promoter sequences of the *PpLOXs* gene family in *P. patens* is shown in Fig. 4. In addition to the core promoter elements CAAT-box and TATA-box, which are common in higher plants, the PpLOXs promoter sequences also contain several special cis-acting elements. These include elements associated with low-temperature response, components involved in abscisic acid, gibberellin, and auxin responses, elements responsive to methyl jasmonate, MYB binding sites involved in drought induction and light response, as well as WRKY transcription factor binding sites. These findings suggest that the PpLOXs gene family may be involved in the growth and development of *P. patens*, as well as in responses to biotic and abiotic stresses.

2.4 Expression analysis of *PpLOXs* gene family at different developmental stages As shown in Fig. 5, except during the sporophyte stage, *PpLOX1* and *PpLOX2* are expressed in all other developmental stages of *P. patens*, with higher expression levels in the gametophyte, protonema, and protoplasts. *PpLOX3* is expressed in all tissues except during the sporophyte S2 and S3 stages, with particularly high expression in the protonema and archegonia. *PpLOX4* is only expressed during the sporophyte SM stage. *PpLOX5* is expressed in the archegonia, spores, and sporophyte S1 and S3 stages. *PpLOX6* and *PpLOX7* are expressed in the gametophyte, protonema, protoplasts, spores, and sporophyte SM stage of *P. patens*. *PpLOX6* shows higher expression in the gametophyte and sporophyte SM stage, while *PpLOX7* exhibits higher expression during the sporophyte SM stage. *PpLOX8* is expressed in the chloronema of the protonema stage and in protoplasts. Therefore, members of the *PpLOXs* gene family are involved in the growth and development of *P. patens* at different stages.

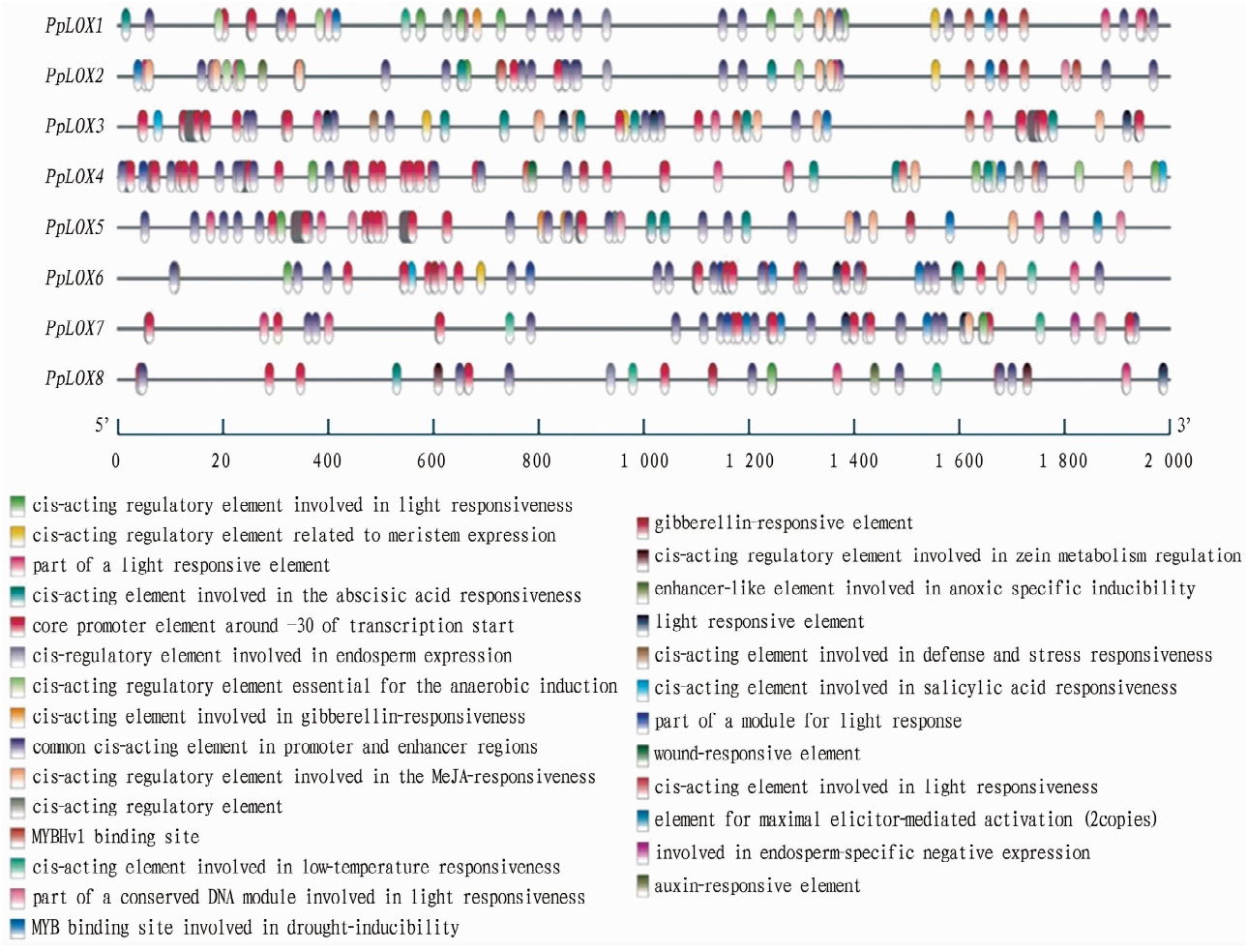


Fig. 4 Cis-acting element of promoter

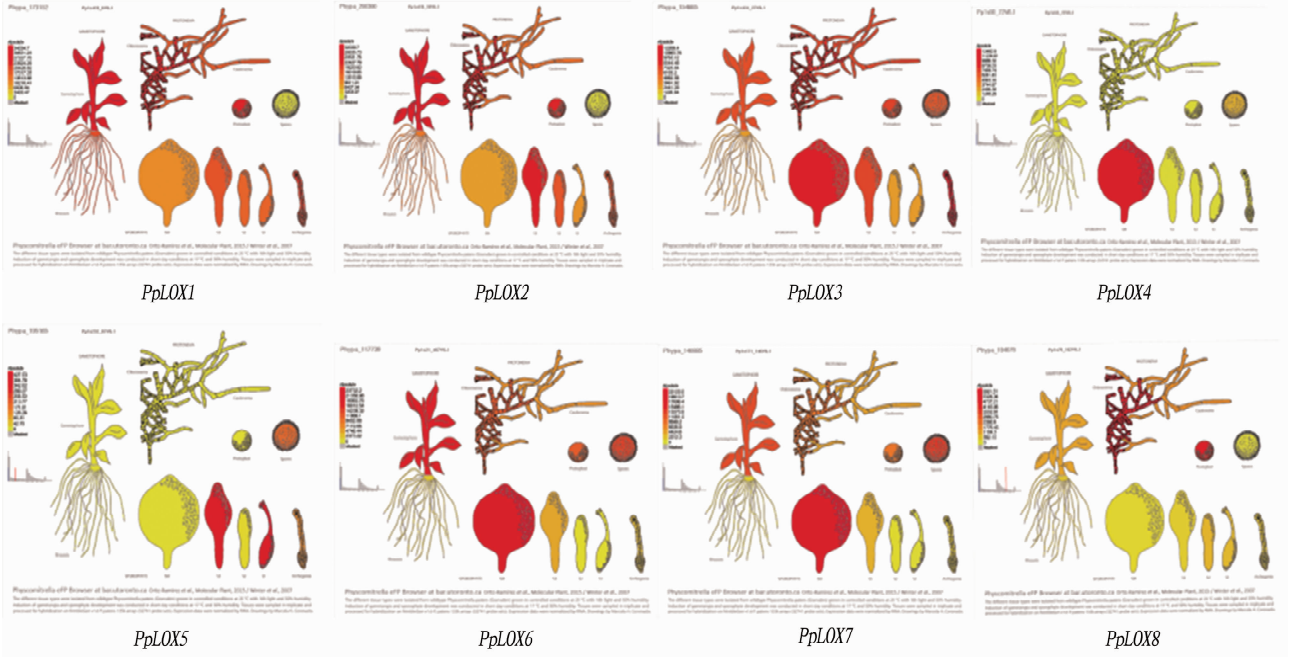


Fig. 5 Expression of *PpLOXs* in different developmental periods of *Physcomitrella patens*

2.5 Expression pattern analysis of *PpLOXs* genes after *B. cinerea infection* As shown in Fig. 6, compared with the control plants (*P. patens* not inoculated with *B. cinerea*), the expression of *PpLOX1* was up-regulated at 2 d after inoculation with *B. cinerea*, and reached its peak at 3 d. The expression of *PpLOX2* was up-regulated at 2 d, peaked at 3 d, with an expression level approximately 18.17 times that of the mock group. The expression of *PpLOX3* was significantly up-regulated at 3 d after inoculation. *PpLOX7* showed up-regulated expression at 3 d post-inoculation, while the expression levels of other family members were lower than those in the control group after inoculation with *B. cinerea*. It is speculated that the lipoxygenase genes *PpLOX1-3* and *PpLOX7* in *P. patens* are involved in the early defense response against *B. cinerea* infection.

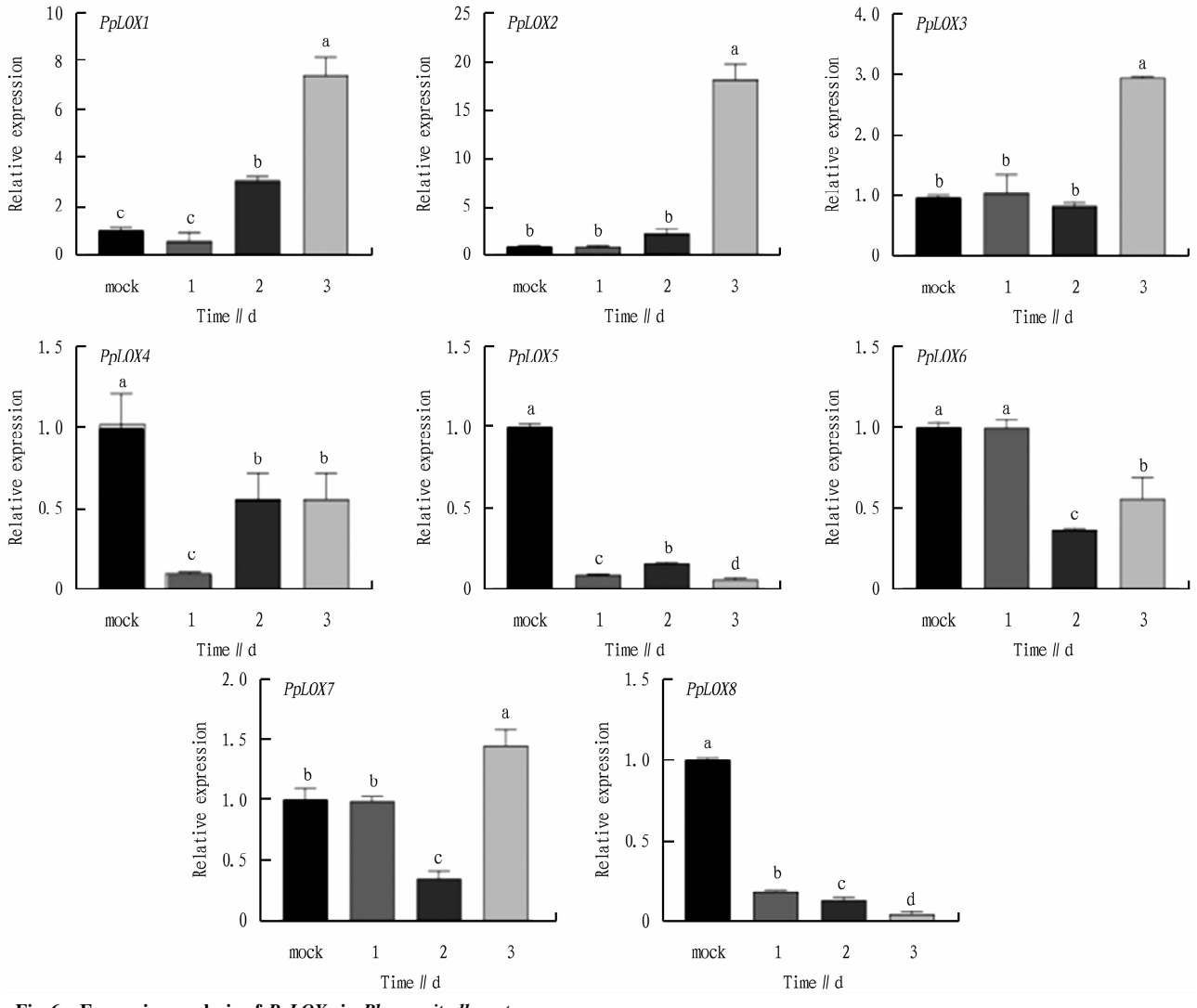


Fig. 6 Expression analysis of *PpLOXs* in *Physcomitrella patens*

3 Discussion

Lipoxygenases (LOXs) are key enzymes in the fatty acid metabolism pathways of plants and animals. Studies have shown that LOXs are involved in plant biotic stress responses[14]. However, the role of the LOX family in the resistance to disease in *P. patens* has rarely been reported.

A profile HMM model was constructed, and SMART screening (http://smart.embl-heidelberg.de/) was employed along with genomic data alignment from NCBI, ultimately identifying eight candidate *LOX* genes in *P. patens*. Phylogenetic analysis revealed that, compared to dicot plants such as *A. thaliana*, the PpLOXs proteins overall share higher homology with LOXs from monocot rice. The evolutionary trajectory from a single *LOX* gene in lower plants like *Porphyra* and *Chondrus* to the expanded *LOX* gene families in higher plants, including bryophytes and dicots, reflects a quantitative increase from few to many. This suggests that LOXs have undergone changes throughout biological evolution, providing evidence for alterations in gene structure and functional differentiation during the early stages of plant terrestrialization.

Most of higher plant LOXs primarily utilize C_{18} fatty acids such as linoleic acid (LA) and linolenic acid (LNA) as substrates, and are classified into 9-LOXs and 13-LOXs based on their

oxygenation positions[15]. Studies have shown that 9-LOXs generally function during plant organ development and fruit maturation[16], while 13-LOXs are mainly involved in defense responses under environmental stress[17]. Chromosomal localization analysis (Fig. 3) revealed that *PpLOX1* and *PpLOX2* are tandemly located in the middle-lower region of chromosome 15, indicating high homology and potentially similar functions. Anterola *et al.*[18] suggested that *PpLOX1* and *PpLOX2* are 12-LOXs, preferentially utilizing 20∶4 and 20∶5 as substrates, which is consistent with previous phylogenetic analyses. *PpLOX5* and *PpLOX8*, as well as *PpLOX3* and *PpLOX4*, exhibit high similarity, while *PpLOX7* shows the lowest similarity with other family members. All of these belong to 13-LOXs and utilize 18∶3 as a substrate[18]. Although *PpLOX6* and *PpLOX1* catalyze different substrates, they share high protein sequence similarity.

PpLOX7 is located on a different branch from other family members, suggesting that the LOXs in *P. patens* have undergone divergence during long-term evolution. The molecular weight and amino acid length of *PpLOX7* are the smallest among the family members, only 71.5 kDa and 640 aa. The pI value of *PpLOX7* is 8.54 (indicating a basic amino acid profile), while other family members are characterized as acidic. This implies that *PpLOX7* may have functionally diverged from other LOXs in *P. patens*, though further investigation is required. Subcellular localization analysis revealed that most *LOX* family members in *P. patens* are localized in the cytoplasm, whereas *PpLOX7* is uniquely localized in the chloroplast. Similarly, Zhu Lili *et al.*[19] reported that among 23 *LOX* genes in *Eucommia ulmoides*, 15 are localized in the cytoplasm and 8 in the chloroplast.

As key enzymes in the fatty acid metabolism pathway of organisms, previous studies have shown that PpLOXs are involved in the growth and development of plants[2]. We found that *PpLOXs* are expressed in all developmental stages of *P. patens*, which is consistent with previous research. The study by León *et al.*[20] demonstrated that pathogens such as *B. cinerea*, *Pythium irregulare*, and *Pythium debaryanum* can induce the expression of *PpLOXs* genes in *P. patens*, although different LOX family members exhibit distinct response mechanisms to different pathogens. For instance, after melon was inoculated with *Fusarium wilt*, the 18 *CmLOXs* gene family members showed varying response times, and their expression also exhibited spatial differences in leaves and roots[21]. This study revealed that after infection of *P. patens* by *B. cinerea*, the eight identified *PpLOXs* genes displayed different expression patterns (Fig. 5). Among them, *PpLOX1-3* and *PpLOX7* were up-regulated to varying degrees. Therefore, *PpLOXs* gene family members may be involved in the early defense response of *P. patens* against *B. cinerea*. Notably, the expression level of *PpLOX2* showed extremely significant differences, and combined with its ability to utilize C20 substrates, it may become a focal point for further in-depth research.

4 Conclusions

In this study, eight *LOX* family genes were isolated and identified from *P. patens*, which were distributed across different chromosomes in a dispersed pattern. Members of the *P. patens* LOX gene family exhibit high evolutionary conservation. Some members of this gene family showed up-regulated expression during the early stages of *B. cinerea* infection, suggesting that *PpLOX1*, *PpLOX2*, *PpLOX3*, and *PpLOX7* may be involved in the early defense response of *P. patens* against *B. cinerea*.

References

[1] QUATRANO RS, MCDANIEL, SF, KHANKELWAL A, *et al.* *Physcomitrella patens*: Mosses enter the genomic age[J]. Current Opinion in Plant Biology, 2007, 10(2): 182 – 189.

[2] RUDOLPH M, SCHLERETH A, KÖRNER M, *et al.* The lipoxygenase-dependent oxygenation of lipid body membranes is promoted by a patatin-type phospholipase in cucumber cotyledons[J]. Journal of Experimental Botany, 2011, 62(2): 749 – 760.

[3] SELTMANN MA, STINGL NE, LAUTENSCHLAEGER JK, *et al.* Differential impact of lipoxygenase 2 and jasmonates on natural and stress-induced senescence in Arabidopsis[J]. Plant Physiology, 2010, 152(4): 1940 – 1950.

[4] MELAN MA, DONG X, ENDARA ME, *et al.* An *Arabidopsis thaliana* lipoxygenase gene can be induced by pathogens, abscisic acid, and methyl jasmonate[J]. Plant Physiology, 1993, 101: 441 – 450.

[5] CAO SX, ZHANG C, TANG YF, *et al.* Protein characteristic of the plant lipoxygenase and the function on fruit ripening and senescence and adversity stress[J]. Plant Physiology Journal, 2014, 50(8): 1096 – 1108.

[6] BANNENBERG G, MARTÍNEZ M, HAMBERG M, *et al.* Diversity of the enzymatic activity in the lipoxygenase gene family of *Arabidopsis thaliana*[J]. Lipids, 2009, 44(2): 85 – 95.

[7] LIAVONCHANKA A, FEUSSNER I. Lipoxygenases: Occurrence, functions and catalysis[J]. Journal of Plant Physiology, 2006, 163(3): 348 – 357.

[8] LI HY, SHI Y, DING YN, *et al.* Bioinformatics analysis of expansin gene family in poplar genome[J]. Journal of Beijing Forestry University, 2014, 36(2): 59 – 67. (in Chinese).

[9] GASTEIGER E, HOOGLAND C, GATTIKER A, *et al.* Protein identification and analysis tools on the ExPASy server[J]. Humana Press, The Proteomics Protocols Handbook, 2005.

[10] BAILEY TL, WILLIAMS N, MISLEH C, *et al.* MEME: discovering and analyzing DNA and protein sequence motifs[J]. Nucleic Acids Research, 2006, 34(S2): 369 – 373.

[11] ORTIZ-RAMÍREZ C, HERNANDEZ-CORONADO M, THAMM A, *et al.* A transcriptome atlas of *Physcomitrella patens* provides insights into the evolution and development of land plants[J]. Molecular Plant, 2016, 9: 205 – 220.

[12] YANG Z, DUAN L, LI H, *et al.* Regulation of heat stress in *Physcomitrium* (*Physcomitrella*) *patens* provides novel insight into the functions of plant RNase H1s[J]. International Journal of Molecular Sciences, 2022, 23(16): 9270 – 9284.

[13] LIVAK KJ, SCHMITTGEN TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) Method[J]. Methods, 2001, 25(4): 402 – 408.

[14] PENG YL, SHIRANO Y, OHTA H, *et al.* A novel lipoxygenase from rice. Primary structure and specific expression upon incompatible infection with rice blast fungus[J]. Journal of Biological Chemistry, 1994, 269: 3755 – 3761.

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identified as one of the primary modes of pertussis transmission. In the adult group, spasmodic cough occurred in only 18.75% of cases, indicating a less prominent presentation of typical symptoms. Pulmonary nodules and fibrotic foci were more commonly observed in the adult group. Therefore, when managing adults with prolonged cough for other pulmonary conditions, clinicians should consider the possibility of pertussis. For children under 3 years of age, clinical manifestations such as facial flushing, cyanosis, vomiting following cough, and cough accompanied by dyspnea/wheezing, often associated with moist rales in the lungs, are significant indicators. Wheezes may also be prominent. These typical clinical presentations facilitate timely diagnosis. However, a subset of cases may present atypically. Clinicians should rely on etiological test results to make an accurate diagnosis promptly and avoid delays in initiating appropriate treatment.

In summary, the following recommendations are proposed:

(i) Enhanced vaccination of family members (cocooning strategy) can provide indirect protection to young infants; therefore, the administration of adult pertussis vaccines should be promoted. (ii) Given that a proportion of pertussis cases lack typical symptoms and are prone to co-occurring pneumonia, leading to potential misdiagnosis during initial presentation, healthcare professional training on pertussis needs strengthening. For suspected cases, prompt nucleic acid testing for *B. pertussis* and testing for Mycoplasma pneumoniae should be performed. Particular attention should be paid to preventing the progression to pneumonia. (iii) Following the adjustments to the Diphtheria – Tetanus – Pertussis (DTP) vaccination schedule implemented in 2025, continuous active surveillance for pertussis should be conducted to understand the evolving epidemiological patterns and clinical characteristics of the disease post-schedule modification.

References

[1] DECKER MD, EDWARDS KM. Pertussis (whooping cough)[J]. The Journal of Infectious Diseases, 2021, 224(S4): S310 – S320.

[2] QIAN ZX, SUN JF. Epidemiology, pathogenesis and treatment advances of pertussis[J]. South China Journal of Preventive Medicine, 2024, 50(4): 296 – 298, 303. (in Chinese).

[3] ZHANG Q, WU D, ZHENG H, *et al.* Epidemiological characteristics of pertussis in China, 2022[J]. Chinese Journal of Vaccines and Immunization, 2024, 30(1): 34 – 38. (in Chinese).

[4] BIN ST, TAN L, WANG J, *et al.* Clinical features and misdiagnosis analysis of 191 children with pertussis[J]. Clinical Misdiagnosis & Mistherapy, 2024, 37(5): 1 – 5. (in Chinese).

[5] ZHANG YQ. Clinical features and clinical prediction model of pertussis in children[D]. Taiyuan: Shanxi Medical University, 2023. (in Chinese).

[6] Diagnosis and Treatment Protocol for Pertussis (2023 Edition)[J]. Chinese Journal of Infection Control, 2024, 23(4): 544 – 546. (in Chinese).

[7] National Health Commission of the People's Republic of China. Diagnosis and Treatment Protocol for Pertussis (2023 Edition)[J]. Electronic Journal of Emerging Infectious Diseases, 2024, 9(3): 86 – 88. (in Chinese).

[8] QIN SM, SONG XL, DONG WY, *et al.* Epidemiological and clinical characteristics of 139 infants with pertussis[J]. Journal of Medical Pest Control, 2022(5): 38. (in Chinese).

[9] YANG YH, LIU XH. Clinical analysis of 56 children with chronic cough [J]. Inner Mongolia Journal of Traditional Chinese Medicine, 2013, 32(33). (in Chinese).

[10] ZHANG J, YOU CF, MING XW, *et al.* Epidemiological characteristics and etiological monitoring of 7 cases of Chlamydia Psittaci in Zigong City [J]. Journal of Preventive Medicine Information, 2025, 5: 436 – 440. (in Chinese).

[11] LI LP, QIU J, SHU XW, *et al.* Pathogen analysis of acute lower respiratory tract infections in hospitalized children in hunan children's hospital in 2013[J]. Practical Preventive Medicine, 2016, 23(10): 1264 – 1268. (in Chinese).

[12] LI XY, YANG XQ, WU JZ, *et al.* Clinical characteristics of 87 hospitalized children with pertussis[J]. China Modern Doctor, 2024, 62(4): 34 – 37. (in Chinese).

[13] ZHAO JJ, LIU YM, ZHAO XY. Current situation analysis and prevention progress of adult pertussis[J]. Journal of Tianjin Medical University, 2024, 30(2): 185 – 189. (in Chinese).

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[15] ESQUERRE-TUGAYE MT, FOURNIER J, POUENAT ML, *et al.* Lipoxygenases in plant signalling[J]. London, Academic Publishers, 1993: 202 – 210.

[16] PORTA H, ROCHA-SOSA M. Plant lipoxygenases. physiological and molecular features[J]. Plant Physiology, 2002, 130(1): 15 – 21.

[17] LÓPEZ MA, VICENTE J, KULASEKARAN S, *et al.* Antagonistic role of 9-lipoxygenase-derived oxylipins and ethylene in the control of oxidative stress, lipid peroxidation and plant defence[J]. Plant Journal, 2011, 67: 447 – 458.

[18] ANTEROLA A, GÖBEL C, HORNUNG E, *et al.* *Physcomitrella patens* has lipoxygenases for both eicosanoid and octadecanoid pathways[J]. Phytochemistry, 2009, 70(1): 40 – 52.

[19] ZHU LL, QING J, DU XQ, *et al.* Genome-wide identification and expression characteristics of *LOX* gene family in *Eucommia ulmoides*[J]. Bulletin of Botanical Research, 2019, 39(6): 129 – 136.

[20] PONCE DLI, SCHMELZ EA, GAGGERO C, *et al.* *Physcomitrella patens* activates reinforcement of the cell wall, programmed cell death and accumulation of evolutionary conserved defence signals, such as salicylic acid and 12-oxo-phytodienoic acid, but not jasmonic acid, upon *Botrytis cinerea* infection[J]. Molecular Plant Pathology, 2012, 13(8): 960 – 974.

[21] SHAO Q, JIE QJ, LIU HJ, *et al.* Effects of LOX activity and gene expression of *Cucumis melo* var. *makuwa* inoculated by *Fusarium oxysporum*[J]. Plant Physiology Communications, 2016, 52(4): 479 – 486.