horticulturae

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Characterization and Expression of Phospholipase D Putatively Involved in *Colletotrichum musae* Disease Development of Postharvest Banana Fruit

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Abstract: Phospholipase D (PLD) in plants plays an important role in growth, development, and stress response. The effect of hexanal on PLD in banana fruit responding to *Colletotrichum musae* infection remains poorly understood. In this study, four putative *PLD* genes, named as *MaPLD1*, *MaPLD2*, *MaPLD3*, and *MaPLD4* were identified from banana fruit. The four *MaPLD*s can be classified into three of the seven known PLD families according to sequence characterization. Their deduced amino acid sequences displayed homology of PLDs from other plant species. Furthermore, the specific expression analysis of *PLD* genes in banana fruit in response to infection in *C. musae* was studied and the response relationship between PLD family members and banana fruit under anthracnose stress was clarified. Changes in both the activity of PLD and PLC, and the connection between hexanal and phospholipases in the banana fruit *C. musae* infection were compared. The results showed that the incidence of disease in banana inoculated with *C. musae* was dramatically increased after 6 days of storage, the activation of PLD and PLC in infected anthracnose fruit before disease development, and that this activation was inhibited by hexanal treatment, which suggested that both enzymes play a protective role in banana fruit to cope with *C. musae* infection and the participation of hexanal in their regulation. Of the four *MaPLD* genes, the anthracnose had a stronger effect on *MaPLD1* and *MaPLD4*. These data demonstrated that hexanal treatment could enhance fruit disease resistance to *C. musae*, and that PLD could take part in the disease defensive system of harvested banana fruit to *C. musae* by modulating the metabolism of cell membrane lipids, and thus suppress disease development in *C. musae* -inoculated banana during storage.

Keywords: phospholipase D; banana fruit; *Colletotrichum musae*; hexanal

Citation: Yi, P.; Li, L.; Sun, J.; He, X.; Li, C.; Sheng, J.; Xin, M.; Ling, D.; Li, Z.; Tang, Y.; et al. Characterization and Expression of Phospholipase D Putatively Involved in *Colletotrichum musae* Disease Development of Postharvest Banana Fruit. *Horticulturae* **2022**, *8*, 312. https://doi.org/10.3390/horticulturae8040312

Academic Editor: Tomasz Piechowiak

Received: 15 February 2022
Accepted: 5 April 2022
Published: 7 April 2022

1. Introduction

Phospholipase D (PLD) catalyzes the hydrolysis of structural phospholipids (e.g., phosphatidylcholine, phosphatidylethanolamine, and phosphatidylglycerol), producing phosphatidic acid (PA) and a free head group. PLD plays important roles in processes of vesicular transport, membrane degradation, and intracellular signaling [1,2]. It is also involved in signaling events that occur in response to a multitude of stimuli, such as freezing, wounding, plant–pathogen interactions, dehydration, and high salinity [3–6]. The PLD gene family has been analyzed in different plant species for nearly two decades. In *Arabidopsis*, a PLD family (*AtPLD*s) can be partitioned into six subgroups, including α-type (3 genes), β-type (2 genes), γ-type (3 genes), δ-type (1 gene), ε-type (1 gene), and ζ-type

(2 genes). Protein structural analysis has revealed several conserved domains in PLDs, including HKD domains, C2 domain, and PXPH domains. All PLDs possess two HKD domains acting as essential catalytic sites [7,8]. Li et al. identified 17 PLD members from *Oryza sativa* through genome-wide analysis and revealed a novel subfamily—the SP-PLD which has a signal peptide instead of a C2 domain or PXPH domain at the N-terminus [9]. Liu et al. found 11 PLD genes in grape and 18 in poplar, and these family members can be divided into six subgroups where the α type formed the largest clade containing 19 genes [10]. In soybean, 18 PLD members (*GmPLD*s) were reported and grouped into six types [11]. In pineapple, based on its genome database, 10 PLD members (*AcPLD*s) were clustered into five types [12]. Lu et al. identified 32 genes encoding PLDs in *Brassica napus* and found high similarity in gene and protein structures existed between *BnaPLD*s and *AtPLD*s [13].

A growing number of studies on PLD functions have been performed in recent years. *OsPLDβ1* from *O. sativa* was reported to be involved in defense response and disease resistance [14], whereas OsPLDβ2 was crucial for controlling ROS-scavenging enzyme at high temperature [15]. Cucumber *PLDα* genes could alleviate the damage of salt stress, and function in the osmotic balancing and structural stabilization of membranes [16]. *Arabidopsis PLD* genes were involved in defense signaling in nonhost resistance against powdery mildew fungi, and *PLDδ* was the main isoform participating in penetration resistance against *Bgh* [17]. The results of *PLDβ1*-knockout and RNAi-suppressed *Arabidopsis* plants indicated that *PLDβ1* played a positive role in the plant response to the necrotrophic fungal pathogen by decreasing expression of JA-biosynthetic and -responsive genes and decreasing JA production [18].

Based on our previous findings, the phospholipase D inhibitor (n-butanol) reduced phospholipase D activity in harvested litchi and longan fruit and inhibited enzymatic browning and oxidative stress during fruit storage [19,20]. Pak Dek et al. [21] reported that tomato fruit treated with the phospholipase D inhibitor (hexanal) showed a marked reduction in PLD activity, as well as the PLD transcript levels. In addition, several studies have demonstrated that aqueous hexanal compositions could effectively extend the shelf life of fruit such as mangos, nectarines, and bananas [22–24]. However, the involvement of PLDs for banana fruit treated with hexanal in disease resistance has not been investigated until now. Previously, we obtained a *PLDα* gene from banana fruit, and preliminarily confirmed that it was closely related to postharvest senescence [25]. The present study was further conducted to clone more typical *PLD* genes from banana fruit, elucidate the influences of hexanal postharvest treatment on enzyme activities, and then investigate their expression characteristics under anthracnose. The results could help improve understanding of the relationship between *MaPLD*s and the senescence deterioration induced by anthracnose in postharvest banana fruit.

2. Materials and Methods

2.1. Plant Materials and Treatments

Banana fruit (*Musa acuminate* L. cv. 'Guijiao No.6', a major commercial variety in China) was harvested from a commercial orchard in Nanning city, Guangxi province of China in August 2019 and 2020. They were immediately transported into Guangxi Key Laboratory of Fruits and Vegetables Storage-processing Technology within 2 h after harvest. Uniform-sized fruit without decay and damage was selected as experimental materials. All experimental materials were immersed for 10 min in 0.1% (*w*/*v*) sodium hypochlorite solution to sterilization and air-dried at room temperature.

These fruits were randomly divided into two groups for postharvest treatments: (a) Banana fruit was dipped for 5 min with 0.5% hexanal dissolved in water containing Tween 20. (b) Banana fruit was dipped for 5 min with sterile distilled water as the control. Each group was applied to three replications, and each replication contained 60 fruits. Then all treated fruit were air-dried overnight and stored at room temperature for *C. musae* infection.

C. musae was isolated from banana fruit infected by typical anthracnose symptoms. The fungus was inoculated, purified on PDA plate, and cultured at 28 °C for 6 days. Banana fruit was sprayed with spore suspension of *C. musae* (10^6 mL^{-1}). All fruit were placed into unsealed plastic bags (0.03 mm thick) and stored at 28 °C for 12 days. Peel tissues were collected on days 0, 3, 6, 8, 10, and 12, frozen in liquid nitrogen, and stored at −80 °C until further analysis.

2.2. Evaluation on Disease Index (DI)

According to the method of Gong et al. [26] with slight modifications, the infected peel area by anthracnose was measured on a score of 0–4, where 0 = no occurrence of disease, 1 = the area of infection affected less than 1/4 of total fruit surface, 2 = the area of infection affected from 1/4 to 1/2 of total fruit surface, 3 = the area of infection affected from 1/2 to 3/4 of total fruit surface, and 4 = the area of infection affected more than 3/4 of total fruit surface. The disease severity index was then calculated as following formula: DI = [Σ (the severity of disease (0–4) × number of fruit with disease)/(maximal disease severity × total number of fruit)] × 100%.

2.3. Cloning of Phospholipase D (PLD) Genes

Total RNA was extracted from 100 mg of frozen banana peels using RNAprep Pure Plant Kit (TIANGEN, Beijing, China). RNA was reverse-transcribed by HiScript®® II Reverse Transcriptase (Vazyme, Nanjing, Jiangsu, China) according to the manufacturer's instructions. A gene fragment based on the conserved region of *PLD* genes from other plant species was amplified using total cDNA as template with two degenerate primers, PLD-DP1 (5′-THGTNATHGTNGAYCAYGARATGCC-3′) and PLD-DP2 (5′-CRCADATDATRTAYTC-RTCRTCNAC-3′). PCR conditions were as follows: 5 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 55 °C, and 90 s at 72 °C, with a final extension at 72 °C for 10 min.

The 5′ end region or 3′ end region was amplified by SMARTer®® RACE 5′/3′ Kit (Clontech, Mountain View, CA, USA) according to the manufacturer's instruction. Four primers (Supplementary Table S1) were designed according to the sequence of internal fragment. The full-length cDNA sequence for *MaPLD* genes were compared to the sequencing results of 3′ RACE, 5′ RACE and internal fragments, and amplified via PCR using four pairs of gene specific primers (Supplementary Table S1). All PCR products were separated through a 1% agarose gel and purified with spin columns (TaKaRa, Dalian, China). The fragments produced were cloned into a pMD19-T vector (TaKaRa) and sequenced by Sangon Biotech Co. (Shanghai, China).

2.4. Determination of MaPLDs Protein Sequences

The deduced amino acid sequence and open reading frame (ORF) of the *MaPLD* genes were searched with the ORF Finder program (https://www.ncbi.nlm.nih.gov/orffinder/ (accessed on 11 September 2019)). Sequence analysis multiple alignments were performed by the National Center for Biotechnology Information (NCBI) and BLAST programs (http://www.ncbi.nlm.nih.gov/BLAST/ (accessed on 11 September 2019)). Physico–chemical properties such as molecular weight, isoelectric point (pI), and the amino acid property were obtained by ExPASy tool ProtParam (http://web.expasy.org/protparam/). The phylogenetic tree was constructed based on MaPLDs protein sequences using the neighbor-joining (NJ) method of MEGA version 7.0 (http://www.megasoftware.net/). Confidence for tree topology was estimated on the basis of 1000 bootstrap replicates.

2.5. Gene Expression Analysis by Quantitative Real-Time PCR (qRT-PCR)

The total RNA was isolated from samples using RNAprep Pure Plant Kit (TIANGEN) according to the manufacturer's instructions. First-strand cDNA was synthesized using HiScript®® II Reverse Transcriptase (Vazyme) following the manufacturer's instructions. Specific gene primers were designed from the cDNA sequences of MaPLD using the Primer 5.0 software (Supplementary Table S1). For qRT-PCR analysis, the clathrin adaptor

complexes medium (CAC) was selected as an internal reference gene [27]. All primers were synthesized by Sangon (Shanghai, China) and listed in Supplementary Table S1. Each qRT-PCR was performed in triplicate with a 20-µL final volume, including 10 µL of 2× ChamQ^{TM}SYBR$^{®®}$Color qPCR Master Mix (Vazyme), 1 µLof cDNA, 0.4 µL of each primer (200 nmol L^{-1} final concentration), and 8.2 µL of ddH_2O in a 96-well reaction plate. PCR conditions were as follows: 95 °C for 5 min, followed by 45 cycles of 15 sat 95 °C, 57 °C for 15 s, and 72 °C for 20 s in Analytik Jena qTOWERE 2.2 (Analytik Jena AG, Jena, Germany). The expression level of the target gene was presented by the fold change [$2^{(-\Delta\Delta Ct)}$] method [28].

2.6. Assays of Enzyme Activities, DAG and PA Content

Banana peels were frozen with liquid nitrogen and ground into powders. One gram of the powder was taken from 20 fruit for analyzing the activities of PLD and PLC according to the method of Li et al. [25] and Shuai et al. [29]. The unit of U g^{-1} was used to express activities on the basis of fresh weight of banana peel. DAG and PA content were quantified according to Shuai et al. [29] with modification. Five grams of banana peel from 20 fruit were used by enzyme-linked immunoreactivity detection.

2.7. Statistical Analysis

Experimental results are presented as the mean ± standard error (SE). Differences among the groups based on a single storage day were evaluated by one-way analysis of variance (ANOVA). The least significant difference at the 5% level was performed using SPSS v17.0 software (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Disease Development

From Figure 1, hexanal-treated fruit showed no sign of disease before 8 days of storage, whereas fungal-treated fruit showed signs of disease spot caused by *C. musae* after 6 days of storage. The disease index reached 1.6 times in fungal-treated samples when the hexanal-treated fruit began to exhibit disease spot on day 8 of storage. The anthracnose disease index of banana progressively increased as storage duration increased. Hexanal treatment resulted in a lower DI than the control, and a noticeable difference was observed between them after 6 days of storage. Hexanal treatment significantly controlled the decay in the harvest banana between 8 and 12 days of storage period, which is similar to that found in tomato [21,30].

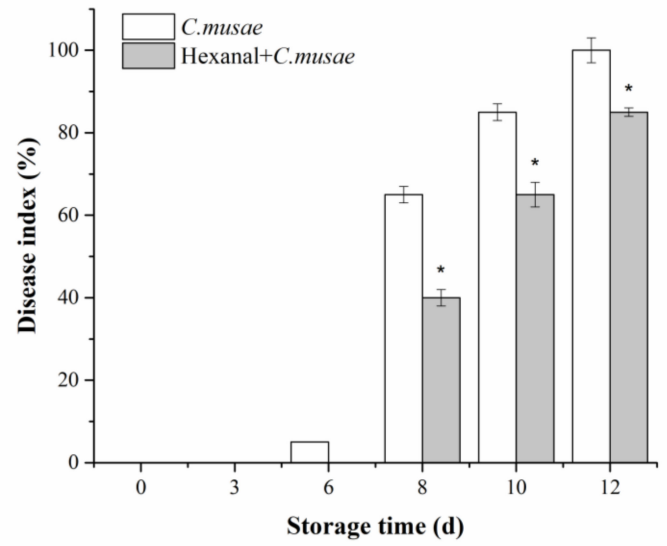

Figure 1. Effect of hexanal treatment on disease index in banana fruits infected with *C. musae*. The experiment was performed in triplicate. Asterisks indicated that values are significantly different between control and hexanal-treated fruits at the same time point using LSD test (* $p < 0.05$).

3.2. Analysis of MaPLDs

Four full-length putative banana PLD genes were identified and named as *MaPLD1*-*MaPLD4*, which has been submitted to the GenBank database (Table 1). The intact ORFs of *MaPLD*s gene were 3120, 2439, 2448, and 1542 bp nucleotides, respectively, predicted to encode proteins of 1039, 812, 815, and 513 amino acid, respectively. The calculated molecular mass was 115.95, 92.57, 92.51, and 57.17 kDa, respectively, and theoretical pI values were 7.48, 5.67, 5.80, and 8.29, respectively.

Table 1. The information of four MaPLD genes in banana.

Name	GenBank No.	C2 Domain	HKD1//HKD2	PLD C Terminal
MaPLD1	MK516209	233–352 aa	+/331 aa/+	962–1030 aa
MaPLD2	MK516210	48–129 aa	+/329 aa/+	733–802 aa
MaPLD3	MK516211	2–153 aa	+/329 aa/+	736–805 aa
MaPLD4	MK516212	-	+/281 aa/+	-

Alignment of the deduced amino acid sequence of the *MaPLD*s clearly showed that their conserved domains contained two conserved HKD motifs (H-x-K-x(4)-D, where x represents any amino acid residue), the 'IYIENQFF' motif, the C2 domain, and phospholipase D C terminal (PLD_C; Table 1 and Figure 2). The two HKD domains were separated by about 330 amino acids, except for *MaPLD4*, which displayed shorter spacing sequences (Figure 2). The C2 domain, the 'IYIENQFF' motif, and the PLD_C were all found among *MaPLD*s, with the exception of *MaPLD4*. The signal peptide between position 1 and 21 was only present in *MaPLD4* but not in other banana PLDs. Alignment analysis indicated that the similarities of the deduced amino acid sequence of the *MaPLD*s varied from 18.7% (*MaPLD3* and *MaPLD4*) to 71.6% (*MaPLD2* and *MaPLD3*).

Based on the presence of domains, four PLD family members in banana were assigned to two main subgroups, C2-PLDs and SP-PLD. C2-PLDs contained the calcium/lipid-binding domain which responsible for regulating Ca^{2+}-dependent activity [7]. Especially, *MaPLD4* encoding an SP-PLD with an N-terminal signal peptide replacing the C2 domain was identified for each of these species. Corresponding SP-PLD genes were also found in a few species, including *OsPLDφ* (Os06g44060, BAD38104.1) from rice [9], *PtPLD11* and *VvPLD3* from poplar and grape [10], *AcPLD9* (Aco008209) from pineapple [12], *GhPLD*]*φ* and *GrPLDφ* from cotton [31].

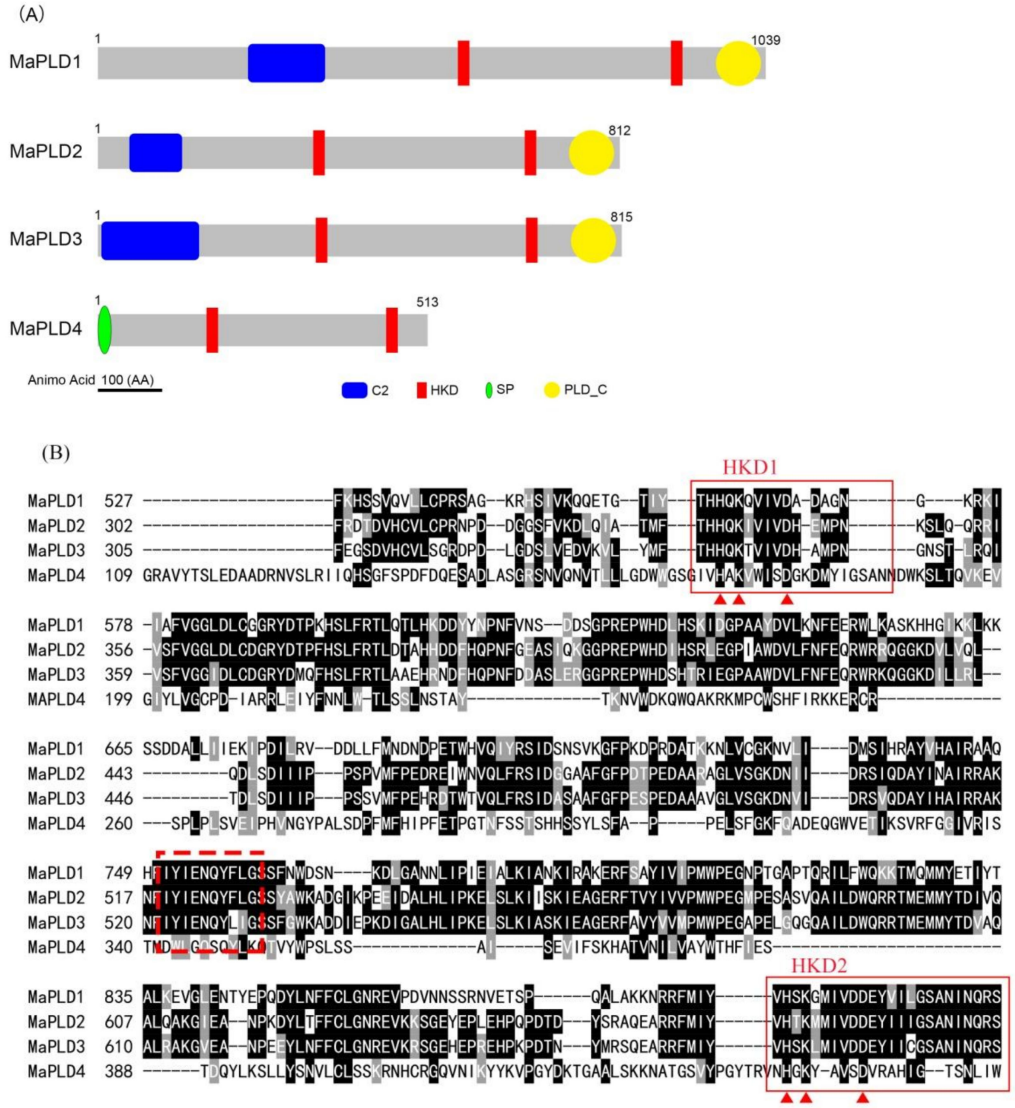

Figure 2. Schematic diagram for domain structures (**A**) and multiple sequence alignment (**B**) of four PLD genes in banana. The C2 domain (protein kinase C-conserved 2 domain), HKD domain, SP (signal peptide) and PLD_C (phospholipase D C terminal) domain are represented by several figures with different colors (**A**). Sequences of two HKD domains were aligned, and the conserved amino acids (His, Lys, and Asp) were marked by red triangles. The 'IYIENQFF' motif was marked by red dotted box (**B**).

3.3. Phylogenetic Analysis of MaPLDs

In order to classify the *PLD* genes gained for banana and investigate their evolutionary relationships, their protein sequences and those of other species were subjected to phylogenetic analysis and a total of 39 *PLD* genes were used in the analysis (Figure 3). The NJ-phylogenetic tree with p-distance and complete deletion option showed that all the *PLD* genes divided into 3 well-supported clades. Among these, the previously classified γ- and β- isoforms clustered closely together and were not explicitly separated from each other. Until now, the whole known plant phospholipase D proteins could be divided into seven types: α, β, γ, δ, ε, ζ, and φ [7–10]. Based on the phylogenetic tree, the four PLD genes were clustered into three types. MaPLD1 was clustered to γ or β type, MaPLD2 and MaPLD3 were α type, whereas MaPLD4 was ζ type (Figure 3). To investigate the potential function of the MaPLD proteins, the transmembrane structure was predicted using TMHMM online tool. The result showed that no transmembrane helix zone existed in the four MaPLDs.

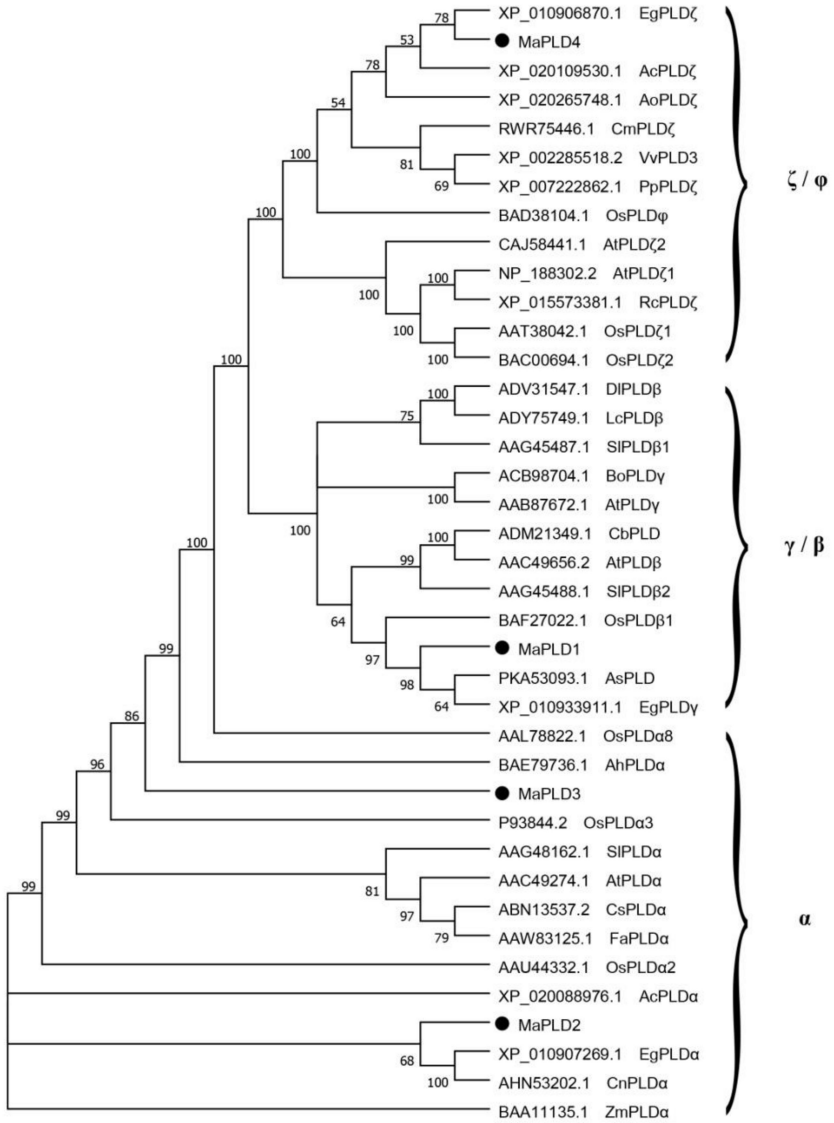

Figure 3. Phylogenetic analysis of *PLD* genes in banana (marked with black solid circles) with other *PLD*s from plants. The NJ tree was constructed by MEGA 7.0. All sequences were labeled with names and GenBank accession numbers.

3.4. Effect of Hexanal Treatment on Expression of MaPLD Genes in Banana Fruit under the Infection of C. musae

To understand the possible role of *MaPLD* genes in banana during the infection of *C. musae*, the expression patterns of *MaPLD1-MaPLD4* in the peel of fruit treated with hexanal were investigated by qRT-PCR. As shown in Figure 4A, with the aggravation of anthracnose infection, the relative expression level of *MaPLD1* (γ-type) significantly showed an increasing trend, and was induced after hexanal treatment, especially on the 10th and 12th day. *MaPLD3* (α-type) showed similar expression patterns with *MaPLD1*. The relative expression level of *MaPLD3* was significantly induced after hexanal treatment, which were 1.90, 1.68, 2.64, 3.34, and 2.89-fold higher than those in the control (Figure 4C). Contrastingly, there was no significant difference in *MaPLD2* (α-type) relative expression between banana fruit treated with hexanal and anthracnose alone (Figure 4B). By contrast, the expression level of *MaPLD4* (ζ-type) in hexanal-treated fruit was decreased by 32% on day 8 of storage when the change of disease index was the most significant (Figure 4D).

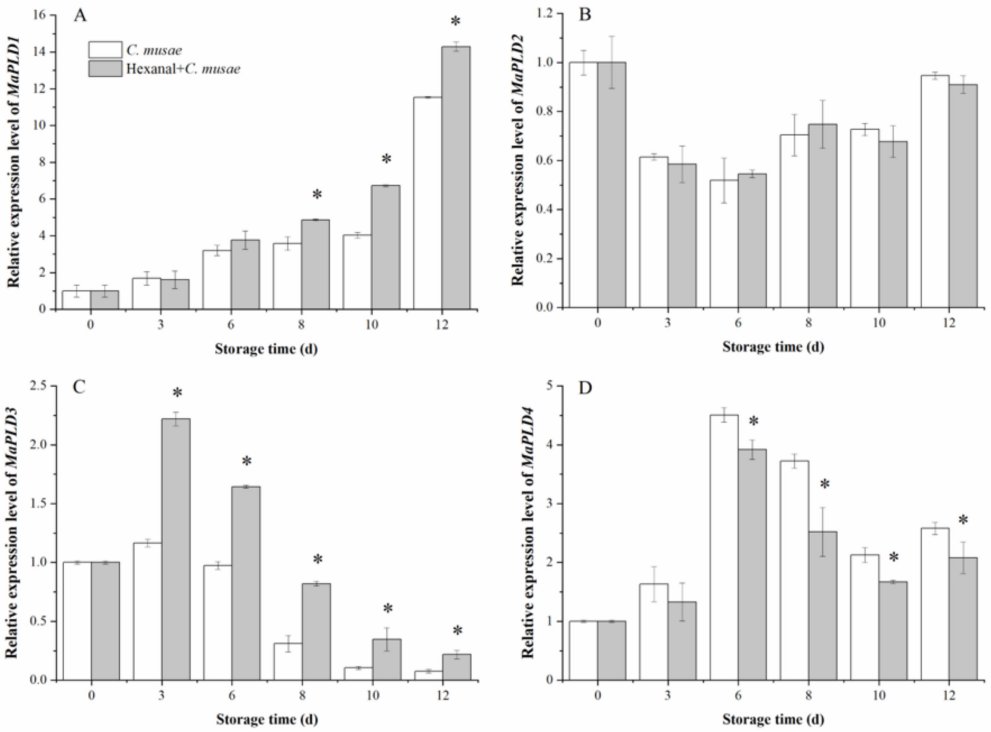

Figure 4. Effect of hexanal treatment on expression of *MaPLD1* (**A**), *MaPLD2* (**B**), *MaPLD3* (**C**), and *MaPLD4* (**D**) genes in banana fruits under the infection of *C. musae*. The experiment was performed in triplicate. Asterisks indicated that values are significantly different between hexanal-treated and *C. musae*-treated fruits at the same time point using LSD test (* $p < 0.05$).

In this study, the *MaPLD*s exhibited differential expression patterns during anthracnose infection of banana. *MaPLD4* was downregulated by hexanal (a PLD inhibitor) treatment, which implied that MaPLD4 protein functioned as a positive regulator during anthracnose infection. The result was similar to the findings of a previous report on *PLDδ* in *Arabidopsis* that showed a positive effect on pathogen defense [17], and a previous report on *AcPLD9* in pineapple that functioned as a positive regulator during internal browning [12]. Notably, *MaPLD1* and *MaPLD3* were upregulated in the presence of hexanal treatment, indicating that MaPLD1 and MaPLD3 protein could be regarded as a repressor in response to anthracnose in banana. Similarly, Hong et al. [12] demonstrated that *AcPLD2* functioned as a repressor in response to hexaldehyde treatment in pineapple. Yamaguchi et al. [14,15] suggested that *OsPLDβ1* and *OsPLDβ2* functioned as a negative regulator of defense responses and disease resistance and involving in the production of chalky grains in high temperature growth conditions in rice, respectively.

According to the results presented, *MaPLD1* may be a key *MaPLD* isoform in protecting banana fruit against the pathogen. The induction of *MaPLD1* in response to infection was greater than that of other *MaPLD* isoforms (Figure 4). The relative expression level of *MaPLD1* gradually increased with the severity of the disease. The involvement of hexanal in the regulation of this gene would appear relevant, especially after outbreak of anthracnose. Interestingly, hexanal treatment significantly induced *MaPLD3* expression in the early stage of anthracnose infection. Our results also showed that *C. musae* induced a marked increase in *MaPLD4* expression levels in response to infection, which suggest that infection favored the phospholipid-signaling mediated by *MaPLD1* and *MaPLD4* in banana fruit, and that hexanal could alter *MaPLD3* expression in some way, whereas the connection between hexanal and the *MaPLD2* isoform in response to infection was barely relevant.

3.5. Hexanal Treatment Induced Changes in Activities of PLD and PLC, and Contents of PA and DAG

In this study, activities of PLD and PLC in *C. musae*-infected banana (Figure 5A,B) followed a similar trend as the disease index (Figure 1), and the increases of PLD and PLC activities were accompanied by the rising banana DI. The data indicated that the enhanced activities of PLD and PLC might lead to phospholipid hydrolysis and lipid peroxidation in cell membranes, resulting in the accelerated disease development of banana fruit during *C. musae* infection, which was in line with the report of Shuai et al. [29]. Compared with the banana fruit with mere inoculation, a lower and slower growth rate of disease incidence was found in hexanal + *C. musae*-treated banana during storage, which was attributed to hexanal-inhibited PLD and PLC activities in *C. musae*-treated banana (Figure 5A,B). PA and DAG were derived from the decomposition of membrane components by PLD and PLC. As displayed in Figure 5C,D, PA and DAG contents in *C. musae*-infected banana increased with the extension of storage period. Contrasted to the fruit with mere inoculation, a slower uptrend of PA and DAG was found in hexanal + *C. musae*-treated banana during storage. Statistical analyses indicated that there were notably ($p < 0.05$) lower activities of PLD and PLC, and notably ($p < 0.05$) lower PA and DAG contents in hexanal + *C. musae*-treated banana than the fruit with mere inoculation during 6–12 d of storage.

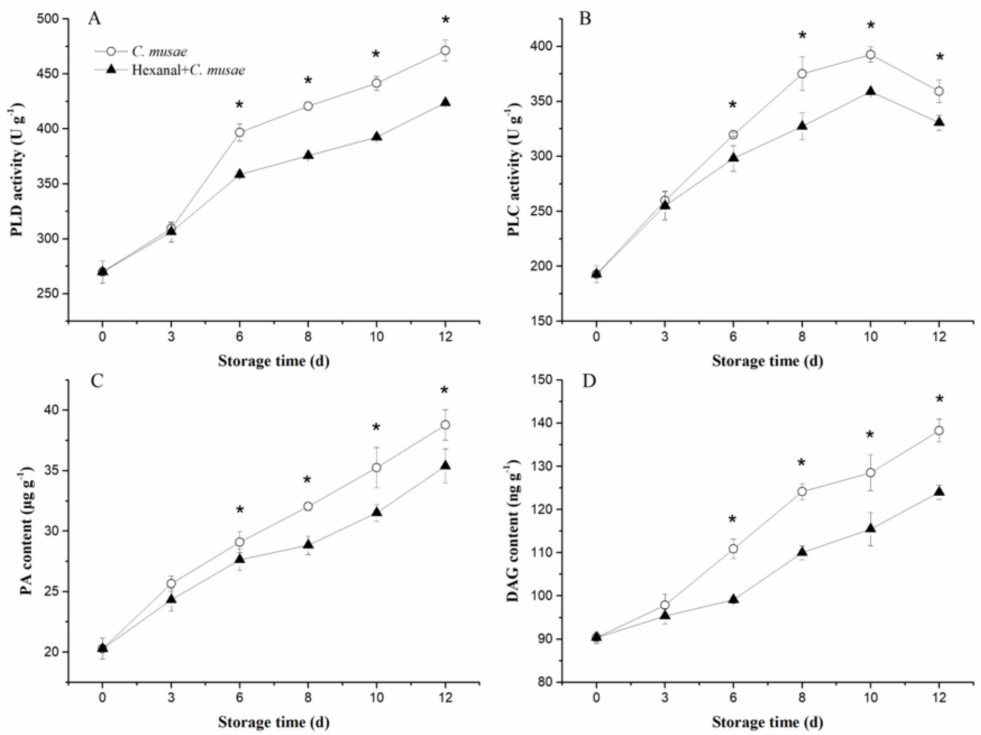

Figure 5. Effects of hexanal treatment on the activities of PLD (**A**) and PLC (**B**), the contents of PA (**C**) and DAG (**D**) in banana fruits inoculated with *C. musae* during storage for 12 days. The experiment was performed in triplicate. Asterisks indicated that values are significantly different between control and *C. musae*-treated fruits at the same time point using an LSD test (* $p < 0.05$).

The above data indicated that hexanal treatment lowered activities of membrane lipid-degrading enzymes (PLD and PLC) and prevented the increases of their degradation products like PA and DAG, implying hexanal treatment could suppress disease development in *C. musae*-infected banana fruit. These findings were in line with the previous studies of Sun et al. [19], Chen et al. [32], and Lafuente et al. [33]. Sun et al. [19] found that n-butanol treatment retarded disease incidence of lichi, which was due to a lower PLD activity. Chen et al. [32] reported that SA treatment could enhance longan fruit disease resistance to *Phomopsis longanae* and suppress disease development in *P. longanae*-inoculated

longans during storage, which owed to the SA-decreasing activities of PLD, PLC, lipase, and lipoxygenase. Lafuente et al. [33] found that the activation of PLD and PLC activities in the *Penicillium digitatum* infected fruit was an early citrus fruit response to cope with pathogen attack stress, and these defense responses could be partially regulated by ABA. Phospholipases have been widely demonstrated to be involved in plant defense against pathogen attack [3]. However, few studies were obtained about fruit crops infected by plant pathogenic fungi, and they focused mainly on the activity of phospholipases rather than in the expression of the different genes encoding them [19,29,33]. In this study, we showed the involvement of different PLDs in banana fruit response to *C. musae* infection in Figure 6.

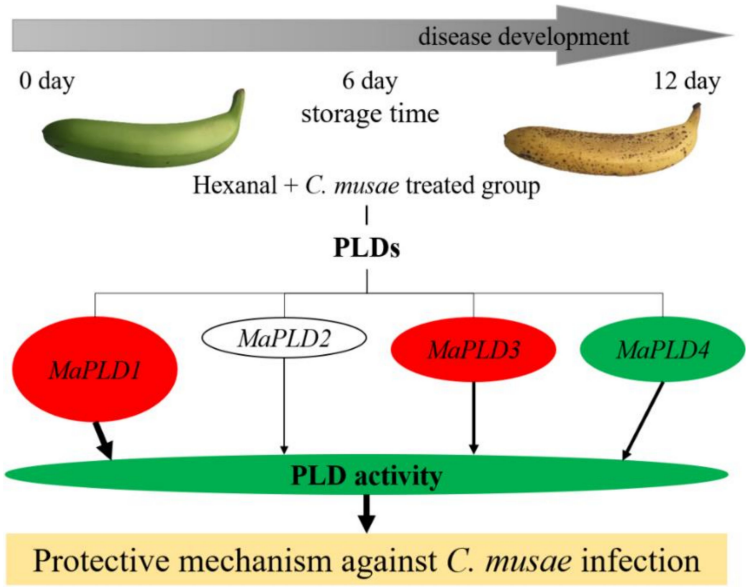

Figure 6. Schematic integration of the changes in *PLDs* gene expression and activity induced by hexanal in banana fruits inoculated with *C. musae* during storage. The effect of hexanal on PLDs gene expression is indicated by shape size: The bigger the size, the more marked the changes. The red color indicated the gene expression was activated, and the green color indicated the gene expression or activity was inhibited. The thicker black arrows indicated more involvement of these genes in banana fruit response to *C. musae* infection.

Anthracnose caused by *C. musae* is one of the most important fungal diseases affecting postharvest banana fruit. The higher the ripeness level of banana fruit, the more serious the disease occurs, which brings great challenges to the storage and transportation of postharvest banana fruit. [34,35]. Therefore, the extension of shelf-life of banana fruit is attributed to the control of postharvest pathogens. At present, some feasible methods have been reported to have a positive inhibitory effect on plant pathogens, especially *C. musae*. Benzothiadiazole or methyl jasmonate treatment significantly controlled the occurrence of anthracnose in postharvest banana fruit, enhanced the activities of defense-related enzymes, and effectively maintained the cell structural integrity [36,37]. In the present study, it has shown that hexanal can be an effective treatment to control the postharvest pathogens and extend the shelf life of banana fruit by regulating PLD activity and gene expression.

4. Conclusions

In summary, four PLD gene family members were identified in banana, characterized based on their conserved domains, phylogenetic analysis, and gene expression profiles during anthracnose infection by qRT-PCR. In this study, the *MaPLD* genes exhibited differential expression patterns during the anthracnose treatment of banana. *MaPLD4* had a positive regulation on anthracnose infection, and *MaPLD1* and *MaPLD3* had a negative regulatory effect. *MaPLD2* did not substantially change among the four genes. PLD in-

duced by anthracnose might stimulate corresponding physiological reactions related to fruit deterioration and senescence through adjusting gene expression. Hexanal treatment alleviated *C. musae*-induced disease incidence of postharvest banana fruit, which involved the inhibition on cellular membrane lipid degradation due to the lowered PLD and PLC activities. This study provided basic information to understand the function of *MaPLD* genes and can help future studies to improve the disease resistance of postharvest banana fruit by regulating PLD activity and *PLD* gene expression.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8040312/s1, Table S1: Primers used to clone the full-length *MaPLD* genes and for qRT-PCR.

Author Contributions: Conceptualization, J.S. (Jian Sun) and L.L.; methodology, P.Y., L.L., X.H. and C.L.; formal analysis, J.S. (Jinfeng Sheng), M.X. and Y.T.; investigation, P.Y., L.L., D.L., Z.L. and G.L.; resources, J.S. (Jian Sun); data curation, J.S. (Jinfeng Sheng), M.X. and Y.T.; writing-original draft, P.Y.; writing-review and editing, L.L.; funding acquisition, J.S. (Jian Sun). All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by National Natural Science Foundation of China (31860579), Guangxi Natural Science Foundation (2020GXNSFAA259040, 2021GXNSFGA196001), China Agriculture Research System of MOF and MARA (CARS-31), Special Fund for Guangxi Bagui Scholars (Grant No. [2016] 21).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: All authors have no conflict of interest.

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