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Full Length Article

Identification of Rice Lipid A Biosynthetic Genes and Expression Profile for Potential Roles in Various Physiological Processes

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Abstract

In most Gram-negative bacteria, 3-deoxy-d-manno-octulosonic acid-lipid A (Kdo_2-lipid A) is the essential component of lipopolysaccharide (LPS) and usually represents the minimal LPS substructure to sustain bacterial viability. Putative genes responsible for lipid A biosynthesis are found in many plants, but little is known about their physiological role. This is the first report on the identification, stress and developmentally regulated expression profiling of lipid A biosynthetic genes in *Oryza sativa*. Rice contains six *lpx* genes, including *OsLpxA*, *OsLpxC*, *OsLpxD*, *OsLpxB*, *OsLpxK* and *OsKdtA*, and many cis-elements in response to stress and development were detected in promoters of these genes. The qRT-PCR analysis showed that *lpx* genes expression was activated early during seedling development (seeds one day after imbibitions). Furthermore, *lpx* genes were constitutively expressed in various types of tissues. Expression of the *lpx* genes was strongly responsive to wounding, and it was slightly responsive to LPS. Transcriptional inhibition of *OsLpxA* promoted the expression of JA-, ET-, or GA synthesis-related genes. Furthermore, transcripts of *OsWRKY71*, *OsMYC2* and *OsNPR1* also increased significantly. These results together suggest that the *lpx* genes play important roles in growth, development and plant defense responses in rice. © 2019 Friends Science Publishers

Keywords: Lipid A; *lpx* genes; Higher plants; Expression profiling; Hormones
Abbreviations: ET, ethylene; GA, gibberellin; JA, jasmonic acid; LPS, lipopolysaccharide; *lpx* genes, lipid A biosynthetic genes; *OsACO4*, ET biosynthetic gene, 1-aminocyclopropane-1-carboxylic acid oxidase; *OsACS2*, ET biosynthetic gene, 1-aminocyclopropane-1-carboxylic acid synthase; *OsAOS2*, JA biosynthetic gene, allene oxide synthase; *OsCPS4*, Camalexin biosynthetic gene, syn-copalyl diphosphate synthase; *OsJMT1*, JA biosynthetic gene, jasmonic acid carboxyl methyltransferase gene; *OsKOL4*, GA biosynthetic gene, ent-kaurene oxidases; *OsKSL8*, Camalexin biosynthetic genes, kaurene synthase-like gene; *OsLpxA*, rice UDP-N-acetylglucosamine acetyltransferase; *OsLpxC*, rice UDP-3-O-acyl N-acetylglucosamine deacetylase; *OsLpxD*, rice UDP-3-O-(3-hydroxymyristoyl)-glucosamine N-acyltransferase; *OsLpxB*, rice tetraacyldisaccharide-1-P synthase; *OsLpxK*, rice tetraacyldisaccharide 4'-kinase; *OsKdtA*, rice 3-deoxy-D-manno-octulosonic-acid transferase; *OsMYC2*, transcription factor; *OsNPR1*, nonexpressor of pathogenesis-related gene; *OsPR1b*, rice pathogenesis-related gene; *OsSAMA1*, ET biosynthetic gene, S-adenosyl-L-methionine synthetase; *OsWRKY71*, transcription factor; qRT-PCR, quantitative real time polymerase chain reaction

Introduction

Lipopolysaccharide (LPS) is the main component of the outer membrane of Gram-negative bacteria and it is a pathogen-associated molecular pattern that induces innate immune responses in animals and plants (Loon *et al.*, 2008; Ranf, 2016; Iizasa *et al.*, 2016). Lipid A is the endotoxic center of bacterial LPS (Llobet *et al.*, 2015). It displays the LPS on the surface of bacterial cells (Rubin *et al.*, 2014). The structure of lipid A among diverse Gram-negative bacteria is relatively conserved (Okada *et al.*, 2016), which usually represents the minimal biologically active unit required for growth. The biological activity of Kdo_2-lipid A is comparable to that of the complete LPS molecule (Raetz and Whitfield, 2002; Raetz *et al.*, 2007).

The Kdo_2-lipid A biosynthetic pathway has been identified in *E. coli*, which is common to most Gram-negative bacteria. Nine enzymes are involved in Kdo_2-lipid A biosynthesis in *E. coli*. Not all Gram-negative bacteria have all nine genes encoding these enzymes (Opiyo *et al.*, 2010). Some Gram-negative bacteria, such as *Acidimicrobium ferrooxidans*, have been found to lack all of the nine genes and others, such as Dictyoglomi, have genes only for the first four enzymes (Lin and Rikihisa, 2003; Opiyo *et al.*, 2010). Most higher plants, including *Arabidopsis thaliana*, *Brassica campestris*, *Oryza sativa japonica*, *Petunia hybrida*, *Ricinus communis*, *Setaria italica*, *Triticum aestivum*, and *Zea mays*, have at least six of the nine *lpx* genes (Opiyo *et al.*, 2010; Li *et al.*, 2011).

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(Opiyo *et al.*, 2010) showed that some or all of the *lpx* genes in higher plants must have been obtained from Gram-negative bacteria. The *lpx* genes of *Arabidopsis thaliana* are functional. The expected lipid A precursors can be detected by mass spectrometry of total lipid when homozygous insertional knockout or RNAi knockdown inactivates the *lpx* genes (Li *et al.*, 2011). Furthermore, many plants, such as green algae, were stained with affinity reagents for Kdo_2-lipid A, implying that lipid A-like molecules can be synthesized in these plants (Armstrong *et al.*, 2006; Opiyo *et al.*, 2010). Despite the presence of *lpx* genes in plants, both the structure and the function of the lipid A-like molecules have not been reported in plants. Although (Joo *et al.*, 2012) suggested that lipid A-like molecules may be involved in plant immune responses or signal transduction because of the low level of these molecules in plants (less than 0.01% of the total lipid), direct evidence is lacking.

We now report the identification of six *lpx* genes encoding *OsLpxA*, *OsLpxC*, *OsLpxD*, *OsLpxB*, *OsLpxK*, and *OsKdtA* responsible for Kdo_2-lipid A biosynthesis in rice. The expressions of the *lpx* genes during rice development and stress response were assessed using quantitative real time PCR (qRT-PCR). We also constructed RNA-interference (Ri) lines targeted at *Oslpx*A. The expression profile of marker genes of hormone-associated pathway was compared between Ri lines and wild type plants. Some defense-related genes were also investigated. We demonstrate that the *lpx* genes play important roles in growth, development and plant defense responses to wounding and biotic stresses.

Materials and Methods

Identification of Rice Lipid A Biosynthesis Genes

To identify *lpx* genes in rice, a PSI-BLAST search of the nonredundant protein sequences database (https://blast.ncbi.nlm.nih.gov/) was conducted using the protein sequences of *E. coli* EcLpxA, EcLpxC, EcLpxD, EcLpxB, EcLpxH, EcLpxK, EcKdtA, EcLpxL and EcLpxM as probes. All putative *lpx* genes identified were subjected to PAPDB database to verify the presence of these genes.

Plant Material and Wounding Treatments

In rice growing season, rice (*Oryza japonica*) seedlings were cultivated in the farm of Changshu Academy of Agricultural Science (Changshu, China) in the spring of 2016. To detect the changes of gene expression in different rice tissues, samples from leaves, sheaths, and roots at four leaf-stage; hulls, stems and young panicles at the heading stage; callus (28 days after induction) were collected for qRT-PCR analysis.

To detect the temporal expression of *lpx* genes during early development, rice seeds were cultivated under a 12 h day/12 h night photoperiod and a 28°C day/22°C night temperature in chambers. Samples from dry seeds, seeds 24 h after imbibitions, plumules 1-day after emergence; plumules 3-days after emergence; plumules 5-days after emergence; radicles 1-day after emergence, radicles 2-days after emergence, radicles 3-days after emergence were collected for qRT-PCR analysis.

To investigate the changes of gene expression in response to wounding stress, rice seeds were grown under 12 h photoperiod for two weeks in chambers. Fully expanded leaves were detached from healthy rice seedlings, cut into strips of about 5 mm, placed on filter paper moistened with Murashige and Skoog (MS) media in Petri dishes sealed with cling film, and incubated at 28°C in the light. The detached leaves without further wounding were incubated as described above and served as non-wounded control. Samples were harvested and frozen in liquid nitrogen at different time points.

Protoplast Isolation and LPS Treatment

Rice seeds were surface-sterilized with 10% sodium hypochlorite, and then grown on half-strength MS medium under light for 3 days. Rice seedlings were then cultured in the dark at 26°C for 14 days. Sheaths of aetiolated young rice seedlings were cut into about 0.5 mm strips. Protoplast isolation from sheaths was carried out according to a previous method (Bart *et al.*, 2006). LPS from *Burkholderia cepacia* were dissolved at 1 mg/mL in water containing 2.5 m*M* $MgCl_2$ plus 1.0 m*M* $CaCl_2$. The working concentration was 100 μg/mL. Protoplasts were treated with 100 μg/mL LPS (water containing 2.5 m*M* $MgCl_2$ and 1.0 m*M* $CaCl_2$ for control), then the protoplasts were collected at 4 h and 8 h after the treatment for qRT-PCR analysis, respectively.

Gene Cloning, Plasmid Construction and Plant Transformation

We had known that UDP-N-acetylglucosamine acyltransferase (EclpxA) catalyzes the first step of lipid A biosynthesis in *Escherichia coli*, so we found OslpxA in rice database by blast with EclpxA sequence. Then the mutant population generated by construction of RNAi plamid. To construct the *OslpxA*-RNAi plasmid, a 207-bp fragment of *OslpxA* CDS was amplified using primers and inserted into the pTCK303 vector. The resulting construct pTCK303-*OslpxA* was introduced into Nipponbare by *Agrobacterium*-mediated transformation to produce RNAi knockdown transgenic lines according to established methods (Hiei *et al.*, 1994). All rice transformants were identified by PCR and qRT-PCR, and the T_2 generations was used for all assays.

Gene Expression Analysis

Rice total RNA was extracted from different tissues using TaKaRa MiniBEST Plant RNA Extraction Kits (TaKaRa, Japan) according to the instructions of the manufacturer.

The quality and integrity of RNA was evaluated by 1.5% agarose gel electrophoresis. RNA samples were quantified using an Agilent 2100 Bioanalyzer, and 5 μg RNA per 50 μL reaction was used to generate cDNA using PrimeScript™ RT Master Mix (TaKaRa, Japan) based on the manufacturer's protocol. qRT-PCR was performed on a ABI 7500 system using a SYBR green detection protocol according to the manufacturer's instructions (TaKaRa SYBR® Premix Ex TaqTM II; TaKaRa, Japan). The rice *UBQ1* gene (Os06g0681400) was used as an internal control. Subsequently, the specificity of each primer pair was confirmed by agarose gel electrophoresis and qRT-PCR melting curve analysis. The relative expression levels of each gene were calculated using the $2^{-\Delta\Delta Ct}$ method (Rao *et al.*, 2013). Two independent biological replicates for each sample were made and each replicate was run three times.

cis-Element Analysis of Promoter Regions of Rice Lipid A Biosynthesis Genes

To identify the various cis-acting regulatory elements in promoters of *lpx* genes, 2500 base pairs (–2.0 kb and +0.5 kb) were analyzed using PlantCARE available online (Higo *et al.*, 1999).

Results

Identification of Lipid A Biosynthesis Genes in Rice

A PSI-BLAST search of the nonredundant protein sequences database was performed using the protein sequences of EcLpxA, EcLpxC, EcLpxD, EcLpxB, EcLpxH, EcLpxK, EcKdtA, EcLpxL, and EcLpxM from *E. coli* as probes. Six nuclear sequences were identified in rice, including OsLpxA, OsLpxC, OsLpxD, OsLpxB, OsLpxK and OsKdtA (Table 1). Homologues of EcLpxH, EcLpxL and EcLpxM were absent in rice, but these are also not found in Arabidopsis and some Gram-negative bacteria. The *OsLpxA* gene encodes two alternative splice variants, the longer transcript *OsLpxA1* encodes a protein with eight additional amino acid residues inserted in the middle region compared to the smaller transcript *OsLpxA2*. The OsLpxA1 and OsLpxA2 are otherwise identical to each other and they all share 38% identity with EcLpxA, with conservation of all active site residues. The *OsLpxC* gene encodes three mRNA splice variants. Studies on EcLpxC had shown that three amino acid residues (H79, H238 and D242) in the C-terminal region of the protein are critical for catalytic activity (Barb and Zhou, 2008). These conserved amino acid residues are found in all protein variants of OsLpxC. Two splice variants of OslpxK (designated OslpxK1 and OslpxK2) were encoded on chromosome 7; these proteins all share 33% identity with EcLpxK, with the presence of Walker A and B motifs, which is required for EcLpxK activity (Emptage *et al.*, 2012). The *OsKdtA* gene encodes two mRNA splice variants designated *OsKdtA1* and *OsKdtA2*. OsKdtA1 shares 34% identity with EcKdtA. OsKdtA2 which lacks exon 3 shares 30% identity with EcKdtA. Single copy genes encoding OsLpxD (LOC_Os07g04200) and OsLpxB (LOC_Os01g54900) were also found, with 38% and 32% identity to EcLpxD and EcLpxB, respectively.

Gene Expression Pattern of Lipid A Biosynthetic Genes

The developmental regulation of *lpx* genes in different tissues was investigated by qRT-PCR. As shown in Fig. 1, all six genes were constitutively expressed in various types of tissues. The expression of *OsLpxD* was the highest in most tissues except for callus and leaves. The *OsKdtA* expression was strongly callus specific, and the expression levels in callus were 6- to 54-fold higher than in other tissues. Interestingly, all *lpx* genes were highly expressed in callus.

Temporal Expression of Lipid A Biosynthetic Genes during Early Development

The qRT-PCR was performed in order to determine the transcript accumulation of *lpx* genes during early seed imbibitions and germination (Fig. 2). The first 24 h after imbibitions of water is crucial for rice seed germination, during which rice embryo cells quickly switch from a metabolically inactive state to a metabolically active state (Bai *et al.*, 2017). Expression changes of six genes which were not expressed in dry seeds, were detected within 24 h of imbibitions, suggesting that *lpx* genes is activated relatively early in rice. All six *lpx* genes started to be highly expressed in plumules and radicles 24 h after germination. In general, *lpx* genes in plumules and radicles tended to have similar expression patterns.

cis-Regulatory Elements in the Promoter of Lipid A Biosynthetic Genes

Promoter analysis was performed on the rice *lpx* genes whose promoter sequences (–2.0 kb and +0.5 kb) were available in the RAP-DB genome database. A total of thirteen development and stress-related regulatory element were selected, including ABA response element (ABRE), anaerobic responsive element (ARE), auxin response element (AuxRR-core or TGA-element), gibberellin response element (GARE-motif), low temperature response element (LTR), myb-binding site (MBS), meristem expression (CCGTCC-box), MeJA response element (CGTCA-motif), heat shock element (HSE), defence and stress response element (TC-RICH), salicylic acid response element (TCA-element), and W-BOX responsible for wounding and pathogen response. Based on this analysis, each *lpx* gene included at least seven cis-regulatory elements in their promoter sequences (Fig. 3). The cis-element CCGTCC-box responsible for meristem-specific

Table 1: Full-length orthologs of *E. coli* lipid A biosynthetic enzymes in rice

Gene names	Locus tags	Splice variants	Protein names	Lengths (Ec/Os)	Identity (%)
OslpxA	LOC_Os01g52400	LOC_Os01g52400.1	OslpxA1	262/327	38
		LOC_Os01g52400.2	OslpxA2	262/319	38
OslpxC	LOC_Os03g12320	LOC_Os03g12320.1	OslpxC1	305/321	35
		LOC_Os03g12320.2	OslpxC2	305/286	36
		LOC_Os03g12320.3	OslpxC3	305/259	34
OslpxD	LOC_Os07g04200	LOC_Os07g04200.1	OslpxD	341/285	38
OslpxB	LOC_Os01g54900	LOC_Os01g54900.1	OslpxB	382/475	32
OslpxK	LOC_Os07g01200	LOC_Os07g01200.1	OslpxK1	328/226	33
		LOC_Os07g01200.2	OslpxK2	328/196	33
OsKdtA	LOC_Os01g63840	LOC_Os01g63840.1	OsKdtA1	425/457	34
		LOC_Os01g63840.1	OsKdtA2	425/416	30

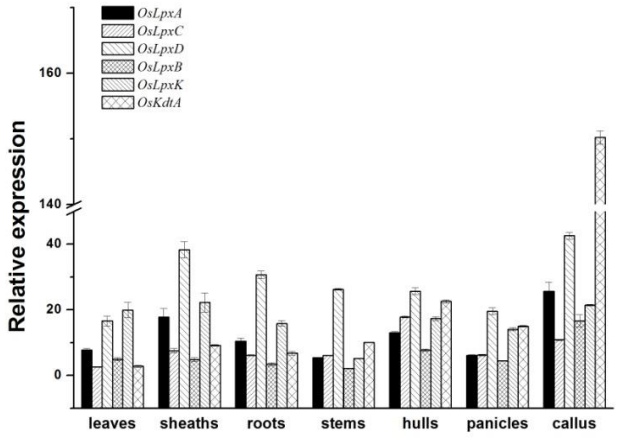

Fig. 1: Expression of *lpx* genes in various tissues. Expression levels were determined by qRT-PCR, the data were normalized according to the transcript level of UBQ1 gene. Each value is the mean of two independent biological replicates, and error bars indicate SD of two biological replicates

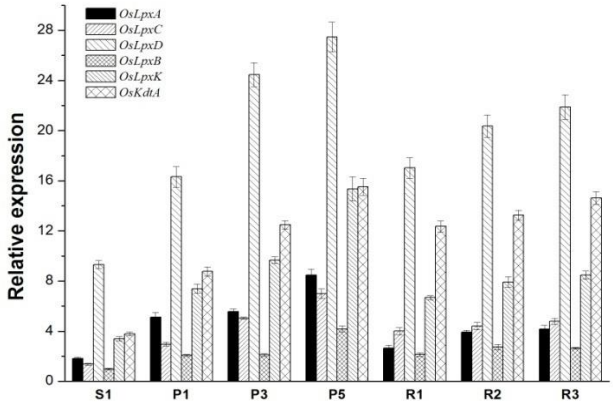

Fig. 2: Expression of *lpx* genes at various early developmental stages. Expression levels were determined by qRT-PCR, the data were normalized according to the transcript level of UBQ1 gene. Each value is the mean of two independent biological replicates, and error bars indicate SD of two biological replicates. S1: seeds one day after imbibitions; P1: plumules one day after emergence; P3: plumules three days after emergence; P5: plumules five days after emergence; R1: radicals one day after emergence; R2: radicles two days after emergence; R3: radicles three days after emergence

expression was identified in all genes, suggesting that these genes were highly expressed in developing tissues. Besides, TC-RICH cis-regulatory element had been identified in the promoter sequence of *OsLpxA*, *OsLpxC*, *OsLpxD*, *OsLpxB* and *OsLpxK* genes, implying that they might involve in stress response. Moreover, it was found that the promoter activity of all *lpx* genes was enhanced by chilling and MeJA.

Stress-induced Expression of Lipid A Biosynthetic Genes

We analyzed the transcriptional changes occurring in rice leaves under wounding stress. Two independent RNA samples were obtained from rice at each stage of stress treatment or from non-treated plants, for the analysis of gene expression profiles. Wound-induced expression data are shown in Fig. 4a. Rice *4CL3*, known to be wound inducible (Sun *et al.*, 2013), was used as a positive control for rice wound treatments, and was up-regulated over 6 fold at 4 h after wounding (Supplementary Fig. S1). Rice *lpx* genes were strongly and transiently up-regulated by wounding. *OsKdtA* was up-regulated 6.6-fold after 4 h wounding, with mRNA reaching below the control levels by 8 h. A similar expression pattern was obtained for *OsLpxD*. *OsLpxA*, *OsLpxC*, *OsLpxB* and *OsLpxK* mRNAs reached maximum levels at 4 h, then decreased gradually, but remained higher than control by 8 h. These data suggest that *lpx* genes participate in defense against wounding.

In gram-negative bacteria, LPS are main components of the outer membrane and are an important microbe-associated molecular pattern (MAMP) that induces various plant defense responses (Newman *et al.*, 2002; Sun and Li, 2013; Finnegan *et al.*, 2016). LPS-induced expression data are shown in Fig. 4b. *OsWRKY71* was a rice *WRKY* gene, was induced by pathogen infection and biotic elicitors (Chujo *et al.*, 2008), was used as a positive control for rice LPS treatment (Supplementary Fig. S2). The *lpx* genes were slightly up-regulated by LPS. These results imply that these genes may participate in the regulation of the responses to biotic stresses.

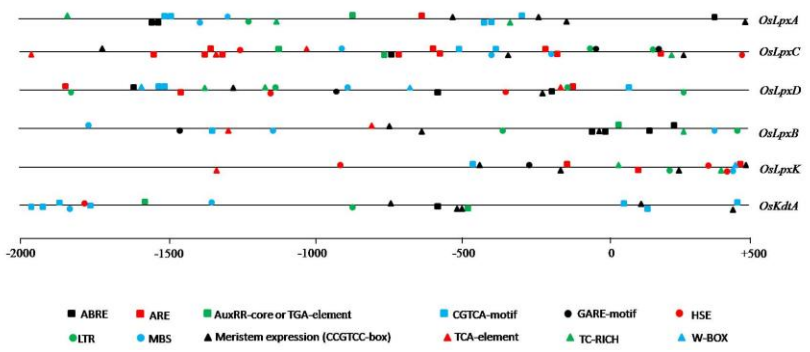

Fig. 3: Promoter analysis of rice *lpx* genes. Regulatory elements in the sense-strand are denoted above the line, and those in the antisense-strand are below

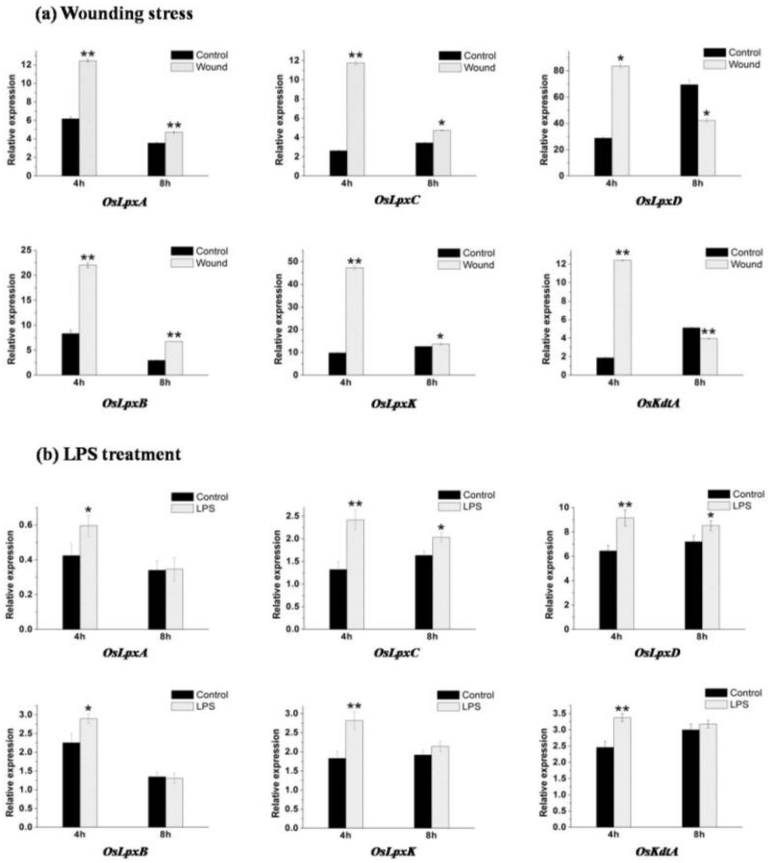

Fig. 4: Expression profiles of *lpx* genes in leaves under various stress treatment. (**a**) Wounding stress. (**b**) LPS treatment. Expression levels of *lpx* genes were determined by qRT-PCR analyses at 4 h or 8 h after treatment. Error bars represent SD ($n = 3$). Asterisks above the bars indicate statistically significant differences compared to control (Student's paired t test: $*P < 0.05$, $**P < 0.01$)

RNAi-mediated Silencing of *OsLpxA* Promoted the Expression of JA-, ET-, or GA Synthesis-related Genes

Rice UDP-N-acetylglucosamine acyltransferase, encoded by *OslpxA*, catalyzes the first step of lipid A biosynthesis. In order to investigate the effects of transcriptional inhibition of *OsLpxA* on rice growth and development, we constructed RNA-interference (Ri) lines targeted at *OslpxA*. Ri lines were viable and showed no obvious phenotypic differences compared to wild-type plants.

To determine whether knockdown of *OsLpxA* affects known defense signaling pathways, the expression profile of marker genes of hormone-associated pathway was compared between Ri lines and

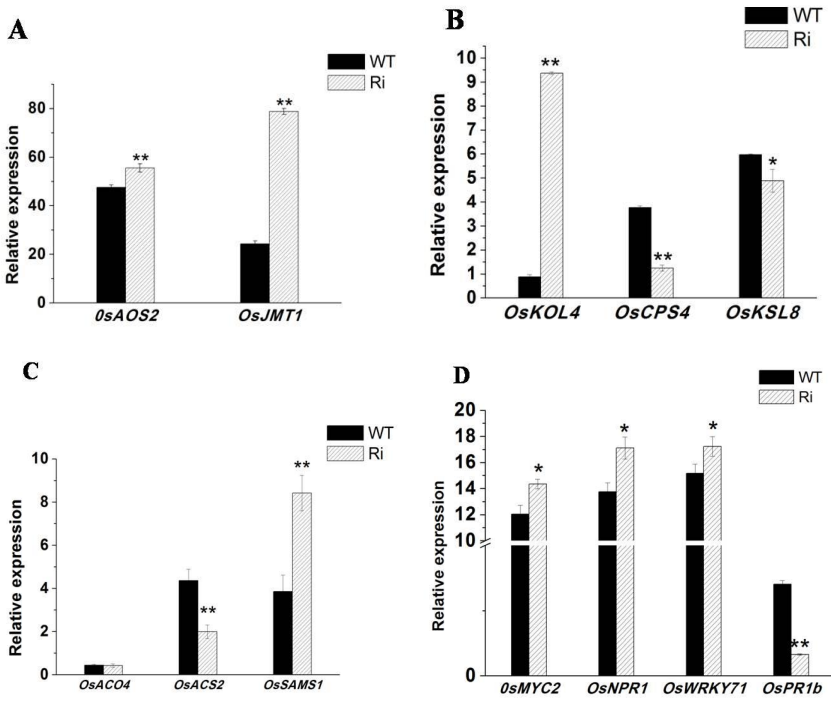

Fig. 5: Defense-related gene expression in leaves from wild-type (WT) and *OsLpxA*-RNAi (Ri) transgenic plants. **A**. JA biosynthetic genes *OsAOS2* (Mei *et al.,* 2006) and *OsJMT1* (Kim *et al.,* 2009); B. GA biosynthetic gene *OsKOL4* (Davidson *et al*., 2004) and Camalexin biosynthetic genes *OsCPS4* and *OsKSL8* (Zhao *et al*., 2016); C. ET biosynthetic gene *OsACO4* (Berre *et al.,* 2017), *OsACS2* (Helliwell *et al.,* 2016) and *OsSAMA1* (Zhao *et al.,* 2017); D. transcription factors *OsMYC2* (Ogawa *et al.,* 2017), *OsWRKY71* (Chujo *et al.,* 2008), nonexpressor of pathogenesis-related gene *OsNPR1* (Yuan *et al.,* 2007), and pathogenesis-related (PR) gene *OsPR1b* (Luan and Zhou, 2015). Error bars represent SD ($n = 3$). Asterisks above the bars indicate statistically significant differences compared to control (Student's paired t test: $*P < 0.05$, $**P < 0.01$)

wild type plants. Some defense-related genes were also investigated. As shown in Fig. 5, transcript abundance of JA synthesis-related genes *OsAOS2* and *OsJMT1*, ET synthesis-related gene *OsSAMS1*, GA synthesis-related gene *OsKOL4*, increased significantly in Ri lines compared to wild-type plants, while those of ET biosynthetic gene *OsACS2*, Camalexin biosynthetic genes *OsCPS4* and *OsKSL8*, and pathogenesis-related (PR) gene *OsPR1b* decreased. Transcripts of stress-related transcription factors, such as *OsWRKY71* and *OsMYC2*, and nonexpressor of pathogenesis-related gene *OsNPR1* increased significantly. We observed no clear change in the expression of ET biosynthetic gene *OsACO4* in Ri lines compared to wild-type plants. The involvement or not of these genes illustrated the complexity of the hormone signaling pathways. Collectively, these results indicate that suppression of *OslpxA* promoted the expression of JA-, ET-, or GA synthesis-related genes and activated the rice defense response.

Discussion

Gram-negative bacteria Kdo_2-lipid A, the minimal viable LPS structure required for growth, is conserved across different species (Raetz and Whitfield, 2002; Raetz *et al.,* 2007; Matsuura, 2013). In *E. coli*, nine enzymes are involved in Kdo_2-lipid A biosynthesis. Many higher plants, including *Arabidopsis thaliana*, have six of the nine enzymes of the *E. coli* system (Li *et al.,* 2011). The *lpx* genes of *A. thaliana* are functional (Li *et al.,* 2011). Arabidopsis *AtLpxA* can replace functionally *E. coli EcLpxA*, complementing an *E. coli* mutant defective (Raetz and Whitfield, 2002; Li *et al.,* 2011). In addition, AtLpxA is able to catalyze the same reaction as EcLpxA *in vivo* (Joo *et al.,* 2012). The expected lipid A precursors can be detected by mass spectrometry of total lipid when chromosomal knockout or RNAi inactivates the *lpx* genes (Li *et al.,* 2011). Although the presence of lipid A genes in plants, the function of the lipid A-like molecules from plants is unknown.

In this study, we revealed that rice contains six *lpx* genes, including *OsLpxA*, *OsLpxC*, *OsLpxD*, *OsLpxB*, *OsLpxK* and *OsKdtA* (Table 1). The upstream sequences of these genes were analyzed using plantCARE database and were found to contain several cis-acting elements, including low temperature responsive elements, dehydration responsive elements, Myb binding sites, meristem-specific expression elements, etc (Fig. 3). These findings suggested that rice *lpx* genes were regulated by both developmental cues as well as diverse stress conditions.

Rice Lipid A Biosynthetic Genes are Essential for Growth and Development

We have showed that the transcript accumulation of rice *lpx* genes was not detectable in dry seeds, but enhanced after imbibitions of water and detected from low level to high level during subsequent germination (Fig. 2), suggesting that these genes were possibly involved in the common biological processes during seed germination. Rice *lpx* genes were expressed in various tissues, and strongly expressed in undifferentiated callus cells (Fig. 1). Furthermore, in-silico analysis of these promoters revealed that all *lpx* genes contained meristematic expression elements (Fig. 3). These findings suggest that the *lpx* genes are essential for rice growth and development.

In this study, we propose that lipid A-like molecules in plants may act as structural components of the outer membranes of mitochondria and chloroplasts. For rice, this proposal is based on the following three observations: (a) The *lpx* genes were highly expressed in various tissues during rice growth and development, but without any apparent tissue specificity (Fig. 1). (b) The mitochondrial localization of the full-length GFP fusion proteins of Arabidopsis *lpx* genes and the accumulation of the lipid X in mitochondria and chloroplasts (Li *et al.,* 2011) implied that lipid A-like molecules of plants might also be localized in mitochondria and chloroplasts. (c) Bacterial LPS containing lipid A is the principal constituent of the bacteria outer membrane. Lipid A-like molecules of plants might have similar structural role.

Lipid A Biosynthetic Genes are Involved in Defense Responses in Rice

In this study, the transcription levels of rice *lpx* genes for lipid A-like molecules biosynthesis were up-regulated by wounding and LPS treatment (Fig. 4). These results imply that the *lpx* genes and their catalytic products lipid A-like molecules involve in plant defense responses. Interestingly, bacterial LPS are major glycolipids of the outer membrane of Gram-negative bacteria known to induce the innate immune response in plants (Keshavarzi *et al.,* 2004; Ranf, 2016; Iizasa *et al.,* 2017). Although the mechanisms by which plants detect bacterial LPS remain unclear (Iizasa *et al.,* 2016), it is necessary for plants to evolve perception of a completely different, truly microbe-specific, LPS substructure to avoid autoimmunity against endogenous LPS-like molecules.

Plant hormones play important roles in regulating plant growth and development under normal conditions and various stresses (Yang *et al.,* 2013; Tuan *et al.,* 2018). We constructed RNA-interference lines targeted at *OslpxA*. These transgenic lines exhibited enhanced activation of hormone synthesis-related genes such as *OsAOS2*, *OsJMT1*, *OsSAMS1* and *OsKOL4*, suggested that rice could mitigate the potential damage of decreased *OsLpxA* mRNA by regulating hormone biosynthesis (Fig. 5). Furthermore, transcripts of *OsWRKY71*, *OsMYC2* and *OsNPR1* increased significantly in Ri lines compared to wild plants (Fig. 5). Collectively, these results also support the hypothesis that the *lpx* genes play vital roles in growth, development and plant defense responses in rice.

Conclusion

In conclusion, we found that rice *lpx* genes were activated relatively early during seedling development (seeds one day after imbibitions) and constitutively expressed in various types of tissues. Expression of the *lpx* genes was strongly responsive to wounding, and it was slightly responsive to LPS. Transcriptional inhibition of *OsLpxA* promoted the expression of JA-, ET-, or GA synthesis-related genes. These results suggest that the *lpx* genes play important roles in growth, development and plant defense responses in rice. The mechanism by which inactivation of *OsLpxA* promotes the expression of JA-, ET-, or GA synthesis-related genes remains unknown and it requires further exploration.

Acknowledgments

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