11. Cloning and expression of *OsLOX1* gene encoding rice lipoxygenase

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Lipoxygenases (LOXs; linoleate:oxygen oxidoreductase, EC 1.13.11.12) comprised a class of non-heme iron-containing enzymes and widely distributed in plants and animals (Siedow 1991, Yamamoto 1992). They catalyzed the addition of molecular oxygen to fatty acids containing at least one (Z), (Z)-1,4-pentadiene system to give their corresponding hydroperoxides (Siedow 1991, Yamamoto 1992, Feussner and Wasternack 2002). In plants, the lipoxygenase pathway of fatty acid metabolism (Vick 1993) was initiated by the addition of molecular oxygen at the C9 or C13 position of linoleic or linolenic acids yielding the corresponding 9- and 13-hydroperoxides (Rosahl 1996). Both 9- and 13-hydroperoxides were subsequently cleaved to short-chain oxoacids and aldehydes by the action of hydroperoxide lyases (HPL) or alternatively, the 13-hydroperoxide was converted, after enzymatic cyclization, reduction, and -oxidation, to jasmonic acid (JA) (Feussner and Wasternack 2002). Schaller et al. (2001) thought that this pathway was parts of plant defense system. In this paper, a novel dual position specific rice lipoxygenase gene (*OsLOX1*) was cloned and then expressed in *E. coli*. The *OsLOX1* transcripts were detected in immature seeds and freshly-germinating seedling with northern blotting at low abundance, further research would be done in future.

To isolate rice seed *LOX* gene, four degenerated primers (LOX-N1, LOX-C1, LOX-N2 and, LOX-C2) were designed according to four conserved regions among C9/C13-LOXs from soybean seed LOX-2 (Genbank accession number J03221), soybean seed *LOX-3* (Genbank accession number X06928) and pea seed *LOX1:Ps:3* (Genbank accession number X78581). RT reaction was conducted with total RNA from the developing seed 10-25 days after flowering (DAF) as template. The first-step PCR was performed with products of RT as template, LOX-N1and LOX-C1 as primers; then the second-step PCR was performed with products of the former PCR as template, LOX-N2 and LOX-C2 as primers. Finally, these nested PCR reactions gave on 1,062 bp product, whose nucleotide sequence was not identical to any previously reported rice *LOX* cDNAs and designated as *OsLOX1*. To further isolate the full length of *OsLOX1* cDNA, we carried out 5′ and 3′ RACE (rapid amplification of cDNA ends). The results showed that the full-length cDNA of *OsLOX1* (GenBank accession number DQ389164) had 3,154 nucleotides and harboured an open reading frame of 2,631 nucleotides (nt), which

encoded a presumptive translation product of 877 amino acid (aa) residues with a *Mr* of 98,754.5 and a *pI* of 7.49. The entire open reading frame (ORF) was flanked by a 284 nt 5′UTR and a 236 nt 3′UTR containing a canonical polyadenylation signal.

Furthermore, the entire ORF of *OsLOX1* was subcloned into an expression vector pET-30a(+) (Novagen) and then expressed in *E. coli* strain BL21(DE3) with IPTG inducer in 20℃ and 37℃. Analysis of the protein from transformed cells by SDS-PAGE showed a detectable supplementary band with the expected molecular weight (Fig. 1). The active protein was present in the soluble fraction of the *E. coli* cell. It showed that the fusion protein acted on both 13-and 9-lipoxygenase, when its products were determined by HPLC (Fig. 2). Genomic DNA isolated from mature leaves were digested with *Eco*RI, *Bam*HI, *Hin*dIII, *Bgl*II, *Nde*I and *Kpn*I, respectively, and transformed to Hybound-N^+ membrane. The hybridization was carried out with a probe made from coding region of *OsLOX1*, and signal detection was performed by ECL^{TM} kit (Amersham) with high stringency. The result displayed that *OsLOX1* had a single copy in rice genome (Fig. 3).

To analysis the expression pattern of *OsLOX1*, we performed northern blotting experiment. Total RNA was also isolated from various tissues and development stages of rice. A specific probe was designed in the *OsLOX1* cDNA fragment (from exon 4 to exon 7) to hybridize *OsLOX1* transcripts. The results showed that *OsLOX1* transcripts were detected in the 20-25 DAF of rice seeds and 1-4 DAI (days after imbibition) of rice freshly-germinating seedling, though their abundances were very low (Fig. 4A and B). Moreover, we found the *OsLOX1* transcripts were undetectable in others tissues or stages of rice (Fig. 4C). Taken together, these results indicated that the *OsLOX1* gene was not a house-keeping gene, and it should be expressed in specific development stage.

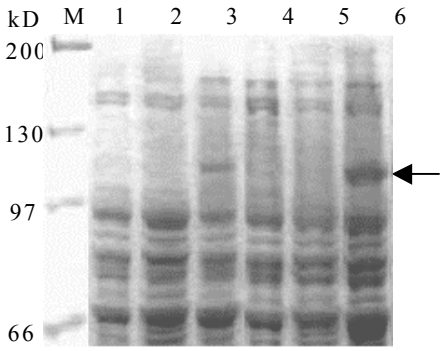

Fig.1. SDS-PAGE analysis of the recombinant proteins. M: High molecular weight marker; Lane 1, 4: total soluble protein of *E.coli*. BL21 (DE3) harboring pET30a(+) induced by IPTG at 20℃ for 3 and 16h, respectively, as control; Lane 2, 5: total soluble protein of *E.coli*. BL21 (DE3) harboring pET30a-OsLOX1 induced by IPTG at 37℃ for 3 and 16h, respectively; Lane 3, 6: total soluble protein of *E.coli*. BL21 (DE3) harboring pET30a-OsLOX1 at 20℃ induced by IPTG for 3, and 16h, respectively; The arrow indicated the target recombinant OsLOX1 protein.

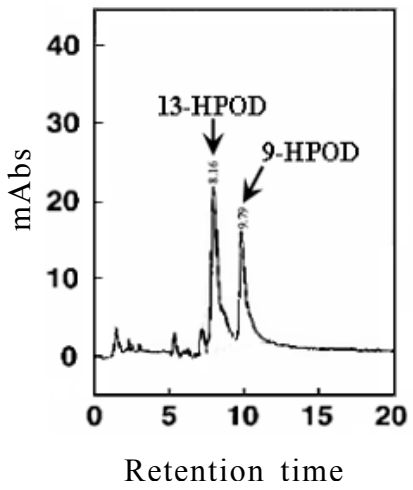

Fig. 2. Determination of positional specificity of the rice OsLOX1. LOX reaction products were reduced by sodium borohydride, acidified to pH 3.0, and extracted with chloroform. Solvent was evaporated, dissolved in methanol, loaded onto a C18 Ultrasphere column (Beckmann, 250 × 4.60mm), and eluted with a methanol/water/acetic acid (85:15:0.1, v/v/v) solvent system. The hydroxylinolenic acids were detected by increase in absorbance at 234 nm.

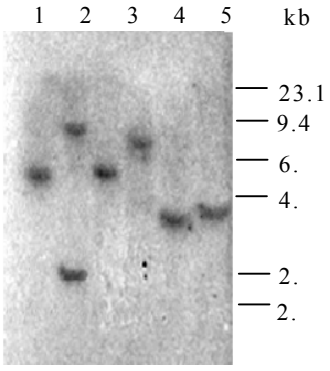

Fig. 3. Southern blot analysis of *OsLOX1*. Lane 1-6: Genomic DNA(10 μg) was digested with restriction enzymes, *Eco*RI, *Bam*HI, *Hin*dIII, *Bgl*II, *Nde*I and *Kpn*I, respecitively.

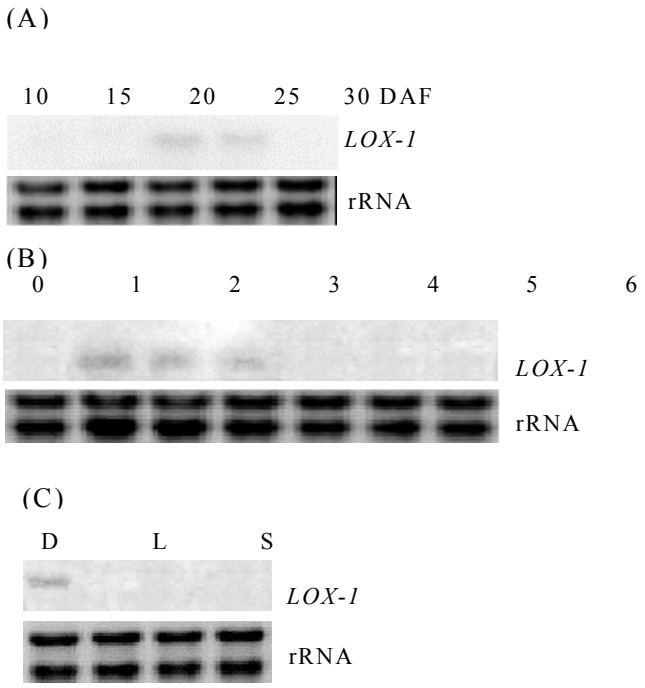

Fig. 4. Gene expression of the rice *OsLOX1* gene in different tissue and developing stage. (A) Developing rice seeds on 10-30 DAF. (B) Seedling on 0-6 DAI. (C) Different tissue of rice. D, developing seeds (15-20 DAF); L, leaves; S, stems; R, roots. Northern blots were performed on total RNAs (30 μg/lane), by using a ^{32}P-labelled probe corresponding to the fragment (665bp) of *OsLOX1* cDNA. Ethidium bromide staining shows equal loading of the RNAs.

References

Feussner I. and C. Wasternack, 2002. The lipoxygenase pathway. Annu. Rev. Plant Biol. Physiol. Plant Mol. Biol. 53: 275–279.

Rosahl S., 1996. Lipoxygenases in plants. Their role in development and stress response. Z Naturforsch 51c: 123–138

Schaller F., 2001. Enzymes of the biosynthesis of octadecanoid derived signalling molecules. J. Exp. Bot. 52:11–23

Siedow J. N., 1991. Plant lipoxygenases: Structure and functions. Annu Rev Plant Physiol Plant Mol. Biol. 42: 145–188

Vick B. A., 1993. Lipid Metabolism in Plants (Moore, T. S., ed) pp. 167–191, CRC Press Inc., Boca Raton, FL

Yamamoto S., 1992. Mammalian lipoxygenases: Molecular structures and functions. Biochim. Biophys. Acta. 1128: 117–131