

Genome-wide analysis of lipoxygenase gene family in Arabidopsis and rice

Pavan Umate*

Department of Botany; Kakatiya University; Warangal, India

Key words: apoptosis, biotic and abiotic stresses, genomics, jasmonic acid, lipids

The enzymes called lipoxygenases (LOXs) can dioxygenate unsaturated fatty acids, which leads to lipoperoxidation of biological membranes. This process causes synthesis of signaling molecules and also leads to changes in cellular metabolism. LOXs are known to be involved in apoptotic (programmed cell death) pathway, and biotic and abiotic stress responses in plants. Here, the members of LOX gene family in Arabidopsis and rice are identified. The Arabidopsis and rice genomes encode 6 and 14 LOX proteins, respectively, and interestingly, with more LOX genes in rice. The rice LOXs are validated based on protein alignment studies. This is the first report wherein LOXs are identified in rice which may allow better understanding the initiation, progression and effects of apoptosis, and responses to biotic and abiotic stresses and signaling cascades in plants.

Lipoxygenases (linoleate:oxygen oxidoreductase, EC 1.13.11.-; LOXs) catalyze the conversion of polyunsaturated fatty acids (lipids) into conjugated hydroperoxides. This process is called hydroperoxidation of lipids. LOXs are monomeric, non-heme and non-sulfur, but iron-containing dioxygenases widely expressed in fungi, animal and plant cells, and are known to be absent in prokaryotes. However, a recent finding suggests the existence of LOX-related genomic sequences in bacteria but not in archaea.¹ The inflammatory conditions in mammals like bronchial asthma, psoriasis and arthritis are a result of LOXs reactions.² Further, several clinical conditions like HIV-1 infection,³ disease of kidneys due to the activation of 5-lipoxygenase,^{4,5} aging of the brain due to neuronal 5-lipoxygenase⁶ and atherosclerosis⁷ are mediated by LOXs. In plants, LOXs are involved in response to biotic and abiotic stresses.⁸ They are involved in germination⁹ and also in traumatin and jasmonic acid biochemical pathways.^{10,11} Studies on LOX in rice are conducted to develop novel strategies against insect pests¹² in response to wounding and insect attack,¹³ and on rice bran extracts as functional foods and dietary supplements for control of inflammation and joint health.¹⁴ In Arabidopsis, LOXs are studied in response to natural and stress-induced senescence,¹⁵ transition to flowering,¹⁶ regulation of lateral root development and defense response.¹⁷

The arachidonic, linoleic and linolenic acids can act as substrates for different LOX isozymes. A hydroperoxy group is added at carbons 5, 12 or 15, when arachidonic acid is the substrate, and so the LOXs are designated as 5-, 12- or 15-lipoxygenases. Sequences are available in the database for plant lipoxygenases (EC:1.13.11.12), mammalian arachidonate 5-lipoxygenase (EC:1.13.11.34), mammalian arachidonate

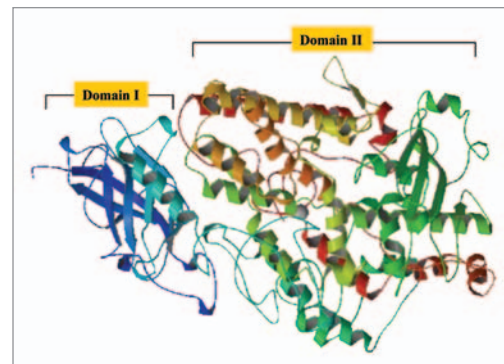


Figure 1. Three-dimensional structure of soybean lipoxygenase L-1. The domain I (N-terminal) and domain II (C-terminal) are indicated. The catalytic iron atom is embedded in domain II (PDB ID-1YGE).²¹

12-lipoxygenase (EC:1.13.11.31) and mammalian erythroid cell-specific 15-lipoxygenase (EC:1.13.11.33). The prototype member for LOX family, LOX-1 of *Glycine max* L. (soybean) is a 15-lipoxygenase. The LOX isoforms of soybean (LOX-1, LOX-2, LOX-3a and LOX-3b) are the most characterized of plant LOXs.¹⁸ In addition, five vegetative LOXs (VLX-A, -B, -C, -D, -E) are detected in soybean leaves.¹⁹ The 3-dimensional structure of soybean LOX-1 has been determined.^{20,21} LOX-1 was shown to be made of two domains, the N-terminal domain-I which forms a β -barrel of 146 residues, and a C-terminal domain-II of bundle of helices of 693 residues²¹ (Fig. 1). The iron atom was shown to be at the centre of domain-II bound by four coordinating ligands, of which three are histidine residues.²²

*Correspondence to: Pavan Umate; Email: pavan_umate@rediffmail.com

Submitted: 09/06/10; Accepted: 09/07/10

DOI: 10.4161/psb.6.3.13546

Table 1. Genes encoding lipoxygenases in *Arabidopsis thaliana* L.

Locus	Annotation	Nomenclature	A*	B*	C*
AT1G55020	lipoxygenase 1 (LOX1)	LOX1	859	98044.4	5.2049
AT1G17420	lipoxygenase 3 (LOX3)	LOX3	919	103725.1	8.0117
AT1G67560	lipoxygenase family protein	LOX4	917	104514.6	8.0035
AT1G72520	lipoxygenase, putative	LOX6	926	104813.1	7.5213
AT3G22400	lipoxygenase 5 (LOX5)	LOX5	886	101058.8	6.6033
AT3G45140	lipoxygenase 2 (LOX2)	LOX2	896	102044.7	5.3177

*A, amino acids; B, molecular weight; C, isoelectric point.

Table 2. Genes encoding lipoxygenases in rice

Chromosome	Locus Id	Putative function	A*	B*	C*
2	LOC_Os02g10120	lipoxygenase, putative, expressed	927	103585	6.0054
2	LOC_Os02g19790	lipoxygenase 4, putative	297	33031.9	10.4799
3	LOC_Os03g08220	lipoxygenase protein, putative, expressed	919	101959	7.4252
3	LOC_Os03g49260	lipoxygenase, putative, expressed	868	97984.5	6.8832
3	LOC_Os03g49380	lipoxygenase, putative, expressed	878	98697.5	7.3416
3	LOC_Os03g52860	lipoxygenase, putative, expressed	871	97183.5	6.5956
4	LOC_Os04g37430	lipoxygenase protein, putative, expressed	798	89304.6	10.5125
5	LOC_Os05g23880	lipoxygenase, putative, expressed	848	95342.9	7.6352
6	LOC_Os06g04420	lipoxygenase 4, putative	126	14054.7	6.3516
8	LOC_Os08g39840	lipoxygenase, chloroplast precursor, putative, expressed	925	102819	6.2564
8	LOC_Os08g39850	lipoxygenase, chloroplast precursor, putative, expressed	942	104494	7.0056
11	LOC_Os11g36719	lipoxygenase, putative, expressed	869	98325.4	5.3574
12	LOC_Os12g37260	lipoxygenase 2.1, chloroplast precursor, putative, expressed	923	104687	6.2242
12	LOC_Os12g37320	lipoxygenase 2.2, chloroplast precursor, putative, expressed	359	40772.7	8.5633

*A, amino acids; B, molecular weight; C, isoelectric point.

This article describes identification of LOX genes in *Arabidopsis* and rice. The *Arabidopsis* genome encodes for six LOX proteins²³ (Table 1). The loci AT1G55020, AT3G45140, AT1G17420 and AT3G22400 are annotated as LOX1, LOX2, LOX3 and LOX5, respectively in the *Arabidopsis* genome database (www.arabidopsis.org) (Table 1). The loci AT1G67560 and AT1G72520 are annotated as “LOX family protein” and “LOX, putative,” respectively (Table 1). These two loci can be considered as LOX4 (AT1G67560) and LOX6 (AT1G72520) (Table 1). The LOX proteins are composed of ~850–930 amino acids (aa) in *Arabidopsis* (Table 1).

Interestingly, the rice genome (rice.plantbiology.msu.edu) encodes for 14 LOX proteins as compared to six in *Arabidopsis* (Tables 1 and 2). Of these, majority of them are composed of ~790–950 aa with the exception for loci, LOC_Os06g04420 (126 aa), LOC_Os02g19790 (297 aa) and LOC_Os12g37320 (359 aa) (Table 2). Four of the LOXs are annotated to contain a chloroplast precursor (Table 2). The Table 3 indicates homology percent identity/similarity for rice LOXs against *Arabidopsis* LOX proteins. The best probable hit with *Arabidopsis* protein database is taken in to consideration while preparing the Table 3. Interestingly, all the rice LOXs showed high level protein similarity with known LOXs from *Arabidopsis* indicating

Table 3. Percent homology of rice lipoxygenases against *Arabidopsis* counterparts

Loci (Os)	Homolog (At)	Identity/similarity (%)	No. of aa compared
LOC_Os02g10120	LOX2	60/76	534
LOC_Os02g19790	LOX5	54/65	159
LOC_Os03g08220	LOX3	66/79	892
LOC_Os03g49260	LOX5	56/73	860
LOC_Os03g49380	LOX5	60/75	861
LOC_Os03g52860	LOX1	56/72	877
LOC_Os04g37430	LOX3	61/75	631
LOC_Os05g23880	LOX5	49/66	810
LOC_Os06g04420	LOX5	49/62	114
LOC_Os08g39840	LOX2	49/67	915
LOC_Os08g39850	LOX2	53/70	808
LOC_Os11g36719	LOX5	52/67	837
LOC_Os12g37260	LOX2	53/67	608
LOC_Os12g37320	LOX2	48/60	160

Os, *Oryza sativa* L.; At, *Arabidopsis thaliana* L.; aa, amino acids.

their novelty at the genomic level (Table 3). The protein alignment shows that LOX sequences are relatively well conserved in rice (Fig. 2).

In plants, programmed cell death (PCD) has been linked to different stages of development and senescence, germination and response to cold and salt stresses.^{24,25} To conclude, this study indicates that rice genome encodes for more LOX proteins as compared to Arabidopsis. The LOX members are not been thoroughly investigated in rice. The more advanced knowledge

on LOXs function might spread light on the significant role of LOXs in PCD, biotic and abiotic stress responses in rice.

Note

αLipoxygenase-mediated modification of insect elicitors has been recently proposed.²⁶ The isolation and expression of the LOX gene indicated its vital role in regulating cell death related to flower senescence, and also in leaf response to phloem feeders in the tea plant.²⁷

References

- Ivanov I, Heydeck D, Hofheinz K, Roffeis J, O'Donnell VB, et al. Molecular enzymology of lipoxygenases. Arch Biochem Biophys 2010; 503:161-74.
- Kühn H, Borngräber S. Mammalian 15-lipoxygenases. Enzymatic properties and biological implications. Adv Exp Med Biol 1999; 447:5-28.
- Maccarrone M, Bari M, Corasaniti MT, Nisticó R, Bagetta G, Finazzi-Agrò A. HIV-1 coat glycoprotein gp120 induces apoptosis in rat brain neocortex by deranging the arachidonate cascade in favor of prostanoids. J Neurochem 2000; 75:196-203.
- Maccarrone M, Taccone-Gallucci M, Meloni C, Cococetta N, Di Villahermosa SM, Casciani CU, et al. Activation of 5-lipoxygenase and related cell membrane lipoperoxidation in hemodialysis patients. J Am Soc Nephrol 1999; 10:1991-6.
- Montero A, Badr KF. 15-Lipoxygenase in glomerular inflammation. Exp Nephrol 2000; 8:14-9.
- Manev H, Uz T, Sugaya K, Qu T. Putative role of neuronal 5-lipoxygenase in an aging brain. FASEB J 2000; 14:1464-9.
- Cathcart MK, Folcik VA. Lipoxygenases and atherosclerosis: protection versus pathogenesis. Free Radic Biol Med 2000; 28:1726-34.
- Feussner I, Wasternack C. The lipoxygenase pathway. Annu Rev Plant Biol 2002; 53:275-97.
- Melan MA, Enriquez A, Peterman TK. The LOX1 gene of Arabidopsis is temporally and spatially regulated in germinating seedlings. Plant Physiol 1994; 105:385-93.
- Gardner HW. 9-Hydroxy-traumatoin, a new metabolite of the lipoxygenase pathway. Lipids 1998; 33:745-9.
- Gfeller A, Dubugnon L, Liechti R, Farmer EE. Jasmonate biochemical pathway. Sci Signal 2010; 3:3.
- Zhou G, Qi J, Ren N, Cheng J, Erb M, Mao B, et al. Silencing OsHI-LOX makes rice more susceptible to chewing herbivores, but enhances resistance to a phloem feeder. Plant J 2009; 60:638-48.
- Wang R, Shen W, Liu L, Jiang L, Liu Y, Su N, et al. A novel lipoxygenase gene from developing rice seeds confers dual position specificity and responds to wounding and insect attack. Plant Mol Biol 2008; 66:401-14.
- Roschek B Jr, Fink RC, Li D, McMichael M, Tower CM, et al. Pro-inflammatory enzymes, cyclooxygenase 1, cyclooxygenase 2 and 5-lipoxygenase, inhibited by stabilized rice bran extracts. J Med Food 2009; 12:615-23.
- Seltmann MA, Stingl NE, Lautenschlaeger JK, Krischke M, Mueller MJ, Berger S. Differential impact of lipoxygenase 2 and jasmonates on natural and stress-induced senescence in Arabidopsis. Plant Physiol 2010; 152:1940-50.
- Bañuelos GR, Argumedo R, Patel K, Ng V, Zhou F, Vellanoweth RL. The developmental transition to flowering in Arabidopsis is associated with an increase in leaf chloroplastic lipoxygenase activity. Plant Sci 2008; 174:366-73.
- Vellosillo T, Martínez M, López MA, Vicente J, Cascón T, Dolan L, et al. Oxylipins produced by the 9-lipoxygenase pathway in Arabidopsis regulate lateral root development and defense responses through a specific signaling cascade. Plant Cell 2007; 19:831-46.
- Axelrod B, Cheesbrough TM, Laakso S. Lipoxygenase from soybeans. Methods Enzymol 1981; 71:441-51.
- Bunker TW, Koetje DS, Stephenson LC, Creelman RA, Mullet JE, Grimes HD. Sink limitation induces the expression of multiple soybean vegetative lipoxygenase mRNAs while the endogenous jasmonate acid level remains low. Plant Cell 1995; 7:1319-31.
- Boyington JC, Gaffney BJ, Amzel LM. The three-dimensional structure of an arachidonic acid 15-lipoxygenase. Science 1993; 260:1482-6.
- Minor W, Steczko J, Stec B, Orwinowski Z, Bolin JT, Walter R, et al. Crystal structure of soybean lipoxygenase L-1 at 1.4 Å resolution. Biochemistry 1996; 35:10687-701.
- Minor W, Steczko J, Bolin JT, Orwinowski Z, Axelrod B. Crystallographic determination of the active site iron and its ligands in soybean lipoxygenase L-1. Biochemistry 1993; 32:6320-3.
- Bannenberg G, Martínez M, Hamberg M, Castresana C. Diversity of the enzymatic activity in the lipoxygenase gene family of *Arabidopsis thaliana*. Lipids 2009; 44:85-95.
- Morel JB, Dangel JL. The hypersensitive response and the induction of cell death in plants. Cell Death Differ 1997; 4:671-83.
- Maccarrone M, Melino G, Finazzi-Agrò A. Lipoxygenases and their involvement in programmed cell death. Cell Death Differ 2001; 8:776-84.
- Vandoorn A, Baldwin IT, Bonaventure G. Lipoxygenase-mediated modification of insect elicitors: Generating chemical diversity on the leaf wound surface. Plant Signal Behav 2010; 5:1674-6.
- Liu S, Han B. Differential expression pattern of an acidic 9/13-lipoxygenase in flower opening and senescence and in leaf response to phloem feeders in the tea plant. BMC Plant Biol 2010; 10:228; DOI:10.1186/1471-2229-10-228.
- Youn B, Sellhorn GE, Mirchel RJ, Gaffney BJ, Grimes HD, Kang C. Crystal structures of vegetative soybean lipoxygenase VLX-B and VLX-D, and comparisons with seed isoforms LOX-1 and LOX-3. Proteins 2006; 65:1008-20.

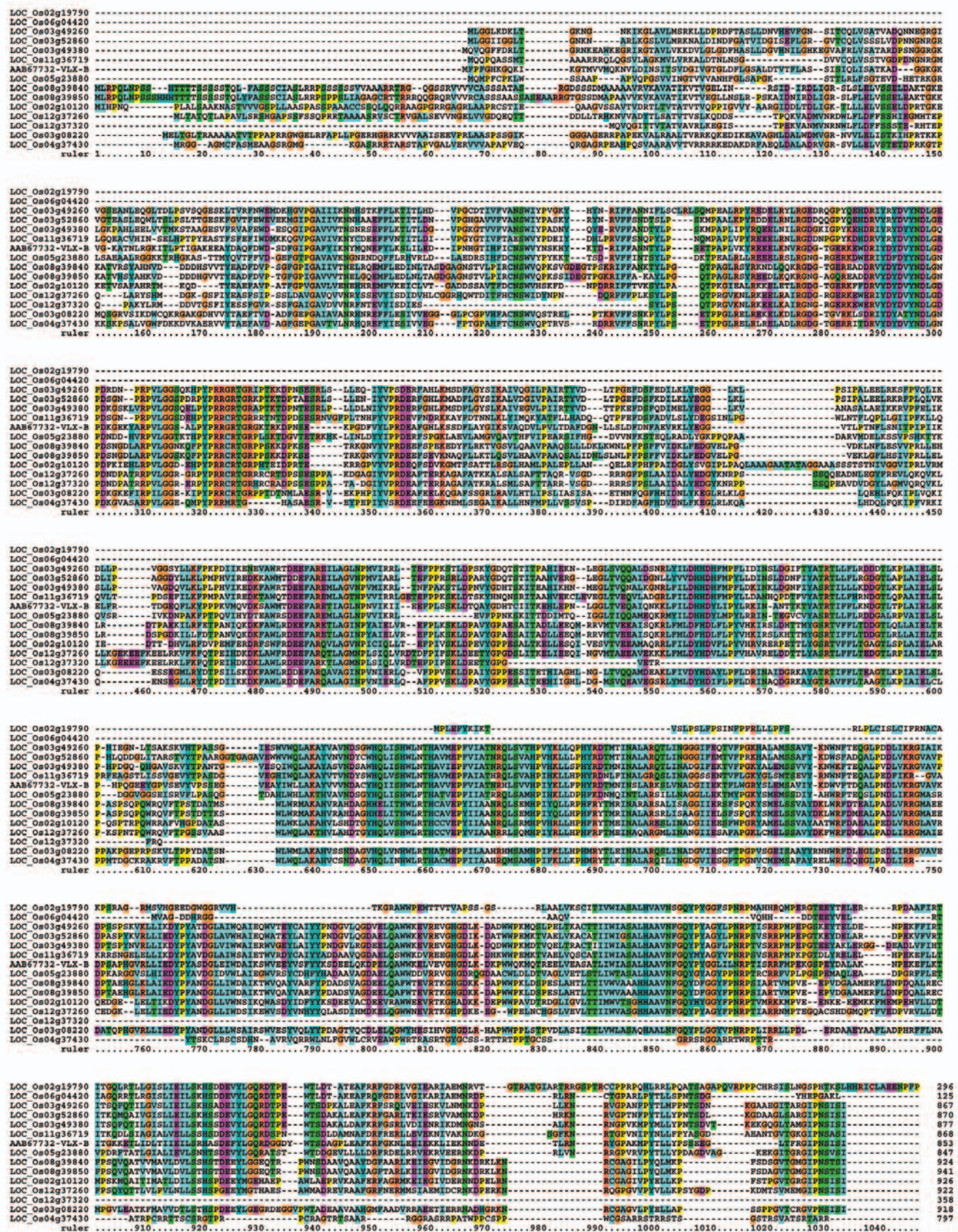


Figure 2. Protein alignment of rice LOXs and vegetative lipoxygenase, VLX-B,²⁶ a soybean LOX (AAB67732). The 14 rice LOCs are indicated on left and sequence position on right. Gaps are included to improve alignment accuracy. Figure was generated using ClustalX program.