Plant Lipoxygenases. Physiological and Molecular Features

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Lipoxygenases (LOXs; EC 1.13.11.12) are nonheme iron-containing dioxygenases widely distributed in plants and animals. LOX catalyzes the addition of molecular oxygen to polyunsaturated fatty acids containing a (*Z*,*Z*)-1,4-pentadiene system to produce an unsaturated fatty acid hydroperoxide. LOX initiates the synthesis of a group of acyclic or cyclic compounds collectively called oxylipins, which are products of fatty acid oxidation, with diverse functions in the cell. In plants, linolenic and linoleic acids are the most common substrates for LOX (Siedow, 1991). Oxygen can be added to either end of the pentadiene system (regiospecificity). In the case of linoleic or linolenic acids, this leads to two possible products, the 9- and 13-hydroperoxy fatty acids (Siedow, 1991). In vitro, most LOXs prefer free fatty acids, though it has been shown that sterified fatty acids are also substrates for LOX in vivo (Feussner et al., 2001; Stelmach et al., 2001), suggesting that membrane lipids could be substrates for oxylipin biosynthesis. The hydroperoxy fatty acid products of the LOX reaction can be further converted to different compounds through the action of enzymes participating in at least six pathways (Fig. 1).

In plants, products of the LOX pathway have several diverse functions (Table I). In addition, LOX has been associated with some processes in a number of developmental stages (Siedow, 1991; Kolomiets et al., 2001), and with the mobilization of storage lipids during germination (Feussner et al., 2001). LOX is also used as a storage protein during vegetative growth (Fischer et al., 1999; Fig. 2).

LOX gene expression is regulated by different effectors such as the source/sink status (Fischer et al., 1999), JA (Creelman and Mullet, 1997), abscisic acid (Melan et al., 1993), and also by different forms of stress, such as wounding (Porta et al., 1999), water deficiency (Porta et al., 1999), or pathogen attack (Melan et al., 1993). In addition, LOX genes isolated from different plant species show differential organ-specific expression (Griffiths et al., 1999; Kolomiets et al., 2001; Table II).

In recent years, our knowledge of the function of LOX and oxylipins in plants has increased with the contributions of many research groups. In addition, a number of plant LOX sequences are now available, making possible the analysis of their phylogenetic relationships and the elucidation of the connections between both LOX sequences and structures and their regiospecificity and activity. The objective of this review is to discuss recent advances on the role of LOXs in the physiology of plants.

DIFFERENT LOXS ARE PRESENT IN THE MATURE SEEDS AND IN GERMINATING SEEDLINGS

LOXs are normally present in the seeds of plants (Siedow, 1991). Nevertheless, LOXs do not have a clear physiological role in seed development, as indicated by the fact that in a soybean line lacking the three seed LOX isozymes, no adverse effects on crop performance were detected when compared with a normal line (Wang et al., 1999). This supports the idea that seed LOXs may function as storage proteins (Siedow, 1991).

In peanuts (*Arachis hypogaea*), the gene coding for PnLOX1 is induced in mature seeds infected with *Aspergillus* spp. (Burow et al., 2000). The products of reactions catalyzed by PnLOX1, namely (13S)-hydroperoxy-(9*Z*,11*E*)-octadecadienoic (13-HPOD) and (9S)-hydroperoxy-(10*E*, 12*Z*)-octadecadienoic acid (9-HPOD), are inhibitor and inducer, respectively, of mycotoxin synthesis, conferring a role in plant-fungus interaction to this particular LOX (Burow et al., 2000).

During germination new LOXs are synthesized in the seedling and the cotyledons. Maximal accumulation of LOX protein and the corresponding mRNAs lasts from a few hours to a few days after germination. The LOX mRNAs synthesized during germination could also be found in the mature plant. Their levels were increased by the application of abscisic acid and JA, or by stresses such as wounding, pathogen infection, or water deficit (Melan et al., 1994; Park et al., 1994; Porta et al., 1999).

In oilseed plants germinated in the dark, storage lipids are mobilized from lipid bodies in the cotyledons, and the free fatty acids that are released are further metabolized via β-oxidation. In germinating cucumber seeds, a specific LOX associated with lipid bodies is capable of adding oxygen to the sterified fatty acids, thus generating triacylglycerol containing one, two, or three 13-HPOD acid residues (Feussner et al., 2001). Oxygenated fatty acids are preferentially cleaved from the lipid bodies and are released into the cytosol (Feussner et al., 2001; Fig. 3).

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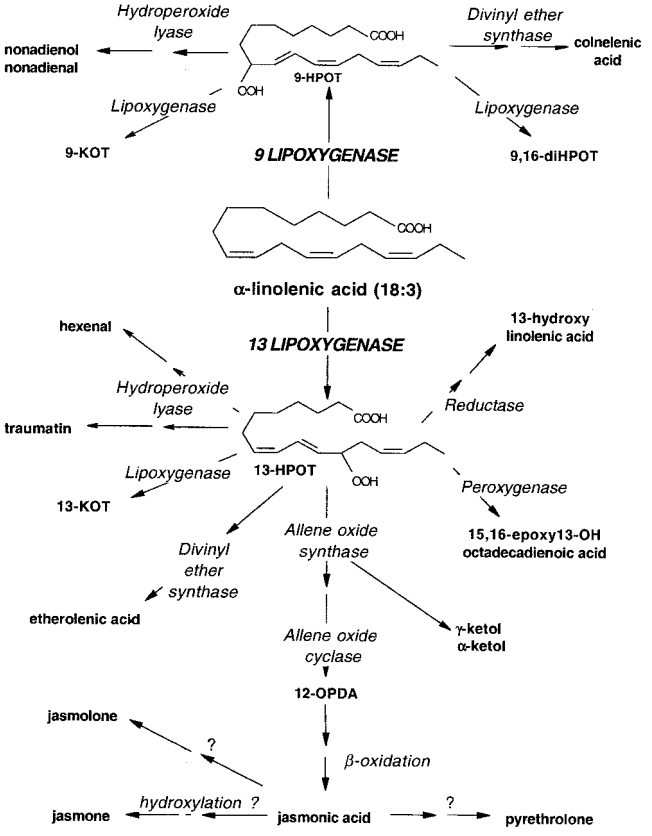

Figure 1. The LOX pathway. The dioxygenation of long chain fatty acids such as α-linolenic acid (18:3), catalyzed by 9- and 13-LOXs, results in derivatives with several known or proposed functions in the plant cell (Table I). 9-HPOT, (10*E*,12*Z*)-9-Hydroperoxy-10,12,15-octadecatrienoic acid; 9-KOT, (10*E*, 12*Z*)-9-keto-10,12,15 octadecatrienoic acid; 13-HPOT, (10*E*,12*Z*)-13-hydroperoxy-10,12,15-octadecatrienoic acid; 13-KOT, (10*E*, 12*Z*)-13-keto-10,12,15-octadecatrienoic acid; 12-oxo-PDA, 12-oxo-10,15-phytodienoic acid.

Three LOXs are present in the mature seed of soybean. Although these isoforms disappear during the first days of germination, three new isozymes are synthesized in the cotyledons. In contrast with the observations in cucumber, seed or seedling soybean LOXs are not associated with lipid bodies. In addition, in germinating soybean seedlings, there is no substantial oxygenation of polyunsaturated fatty acids. This suggests that in soybean, LOX is not used for lipid mobilization during germination (Wang et al., 1999).

LOXS ARE INVOLVED IN VEGETATIVE GROWTH

The production of transgenic plants expressing an antisense, tuber-specific LOX (POTLX-1) gene gave some clues about the function of LOX in potato tubers (Kolomiets et al., 2001). Based on its deduced amino acid sequence, POTLX-1 belongs to class 1 of LOXs in potato, which are expressed in tubers and roots and have predominantly LOX-9 activity. In situ localization showed that Lox1 class mRNA is found in the distal, most actively growing portion of the developing tuber. Antisense POTLX-1 plants displayed reduced LOX activity and a severalfold reduction in tuber yield. Tubers that formed were misshapen and small. These results suggest that LOXs are involved in the control of tuber growth and development, probably by initiating the synthesis of oxylipins that regulate cell growth during tuber formation (Kolomiets et al., 2001).

In legume nodules the presence of LOX proteins and mRNAs have been reported in several species. In *P. vulgaris* nodules, LOX mRNAs and proteins are detected mainly in nodules in the growing stage, and their levels decrease in nodules that have reached their full size. LOX antigen is found in the nodule parenchyma and in the uninfected cells of the central nodule tissue. Most likely, this pattern of accumulation is associated with nodule development (Porta et al., 1999).

In tomato three different LOX mRNAs, corresponding to the nuclear genes encoding TomloxA, TomloxB, and TomloxC, are active during fruit ripening. TomloxC is a chloroplastic LOX. These genes are differentially regulated during fruit ripening and their expression is affected by ethylene and unknown developmental factors (Griffiths et al., 1999). In addition to a possible defense function, fruit LOXs could be involved in the synthesis of the C6 aldehydes responsible for flavor and aroma of the tomato fruit, or in the degradation of thylakoid membranes during the transition from chloroplast to chromoplast (Griffiths et al., 1999).

LOX IS USED AS A VEGETATIVE STORAGE PROTEIN (VSP)

Non-seed tissues synthesize storage proteins termed VSPs that are different from the storage proteins found in seeds. In soybean leaves, VSPs are found in the vacuoles of the bundle sheath (BS) and in paravenial mesophyll (PVM) cells. VSPs are also present in flowers, germinating cotyledons, and pod walls. VSP genes are regulated during development as a function of the need to store excess nitrogen, and the accumulation of their products responds to source/sink relationships. VSP gene expression is enhanced by sink limitation (pod or shoot tip removal), high nitrogen, water deficit, wounding, and JA (Staswick, 1990; Fischer et al., 1999). Three soybean VSPs, namely VSP27 (VSPα), VSP29 (VSPβ), and VSP94, have been characterized. VSP94 is a member of the LOX family (Tranbarger et al., 1991). More detailed studies have revealed that at least five vegetative lox proteins (VLXA, VLXB, VLXC, VLXD, and VLXE) accumulate to high levels in soybean leaves in response to sink limitation (Fischer et al., 1999). VLXA, VLXB, and VLXC are located in the cytosol of PVM cells and, after depodding, they also accumulate in the cytosol of BS and adjacent cells. By con-

Table I. *Products of LOX metabolism with a known or proposed activity*

Compound	Branch	Activity
(13S)-Hydroperoxy-(9Z-11E)-octadecadienoic (13-HPOD) and HPOT	–	Inhibitors of mycotoxin synthesis[a]
9- and 13-HPOD or HPOT	–	Development of hypersensitive cell death[a]
Jasmonic acid (JA)	Allene oxide synthase (AOS)	Signaling in several stresses and tendril coiling[a]
OPDA	AOS	Signaling in wounding and pathogen attack[a]; tendril coiling[a]
(C6-) volatiles (aldehydes and alcohols)	Hydroperoxide lyase (HPL)	Signaling in wounding[a]; attractors to enemies of herbivores[a]; antimicrobial[a]; odors[a]
Dinor-oxo-phytoclienoic acid	AOS	Signaling in wounding[a]
9- and 13-ketodienes	LOX	Signaling in wounding and pathogen attack[b]; induction of cell death[b]
Traumatin	HPL	Signaling in wounding[a]
(Z)-jasmone	AOS	Herbivore repellent and attractor of enemies of herbivores[a]; signaling in plant defense[a]
Colneleic and colnelenic acids	Divinyl ether synthase (DES)	Antifungal[a]

[a] Known activity. [b] Proposed activity.

trast, after sink limitation, VLXD accumulates in the vacuole of BS and PVM cells, suggesting that this is a major storage protein in soybean leaves. Other VLXs (VLXA, VLXB, and VLXC) may function during assimilate retranslocation through the PVM cell layer, as active enzymes in lipid metabolism and/or membrane reorganization (Fischer et al., 1999).

Soybean pod walls function as a major nutrient reservoir for developing seeds. They accumulate high amounts of LOX and VSPα during development, and these proteins are the first to diminish during seed filling (Dubbs and Grimes, 2000b). VLXD is the main storage form of the VLX protein in this organ: Before seed filling, VLXD accumulates mainly in the vacuoles and cytoplasm of the endocarp middle zone (Dubbs and Grimes, 2000b). In contrast, VLXA, VLXB, and VLXC are localized in the cytoplasm of cells of the mid-pericarp cell layer (MPL), a single discrete layer in the mesocarp. MPL cells are larger and more branched than adjacent cells, and form a network with tight-fitting interconnections. Extensive regions of the cell wall of MPL cells are thin and occasionally break, allowing mixing of cellular components (Dubbs and Grimes, 2000a). When a pod wall is disrupted, a LOX-enriched exudate appears to emanate from the MPL. It has been speculated that this cellular disruption could bring LOX into contact with its substrate, thus triggering the LOX-associated defense response (Dubbs and Grimes, 2000a).

DIFFERENT OXYLIPINS ARE REQUIRED FOR THE DEFENSE OF PLANTS

Wounding and Herbivore Attack

The induction of LOX transcripts in wounded and systemic leaves in the same plant has been observed in several species after mechanical wounding or insect feeding. The function of LOX in wounding seems

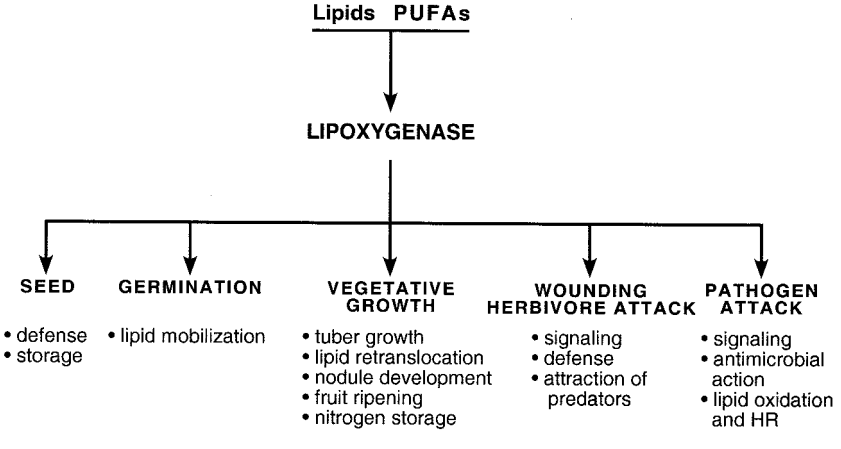

Figure 2. LOXs have active roles in several processes during plant life. PUFAs, Polyunsaturated fatty acids.

Table II. *Plant LOX genes in different species: regiospecificity and subcellular localization of corresponding LOXs, and expression patterns of their mRNAs*

Plant	LOX Name	Accession No.[a]	Regiospecificity[b]	Expression[c]
Arabidopsis	Lox1	L04637		G, P, ABA, J, L, R, In
	Lox2[d]	L23968		L, J, W, G, In
Peanut (*Arachis hypogaea*)	PnLOX1	AF231454	13/9 (70/30%)	S, J, W, P
Cucumber (*Cucumis sativum*)	CSLBLOX[e]	CAA63483	13/9 (84/16%)	G
	CRLOX-1	U36339	13/9 (54/46%)	R
Soybean (*Glycine max*)	L-1	J02795	13/9 (95%/5%)	S, Ws
	L-2	J03211	13/9 (50/50%)	S
	L-3	X06928	13/9 (50/50%)	S, Ws
	LOXA[f]	U04785		R, L, G, J, Au, Po, VSP
	LOXB[f]	U50075		L, Fl, W, VSP, Po
	LOXC[f]	U26457		L, VSP, Po
	LOXD[g]	U04526	13/9 (80/20%)	R, G, L, Au, Po, VSP, J
Barley (*Hordeum vulgare*)	LoxA[f]	L35931		S, G, J, W
	LoxC[f]	L37358		S, G, J, Ws
	Lox2:Hv:1[d]	U56406	13 (89/11%)	J, SA
Lentil (*Lens culinaris*)	Lox1	X71344	13/9 (82/18%)	
Tomato (*Lycopersicum esculentum*)	LoxA	U09026		F, G, J
	LoxB	P38416		F, Et
	LoxC[d]	U37839		F, Et
	LoxD[d]	U37840		L, W, J, Sy, Fl
Tobacco (*Nicotiana tabacum*)	Lox1	X84040		E, P, J
Rice (*Oryza sativa*)	L-2	X64396	13	G
	Lox2:Os:1	D14000	13	P
	RCI-1[d]	AJ270938	13 (99/1%)	BTH, INA, J
Common bean (*Phaseolus vulgaris*)	LOX1	X63525		Fl, St, L
	PvLOX2	U76687		N, L, R, W, J, Fl, S
	PvLOX5	AF234983		N, R
Pea (*Pisum sativum*)	LOX1:Ps:1	AF098918		G, R, N
	LOX1:Ps:2	X78580	13/9 (88/12%)	S
	LOX1:Ps:3[f]	X78581	13/9 (44/66%)	S
Potato (*Solanum tuberosum*)	POTLX-3	U60201		J, Et, P
	H1	X96405	13	L, W, J, ABA
	H3	X96406	13	L, W, J, R, ABA
	T8	X95513	9	T, R, W, J
	lox1:St:2	Y18548	13/9	L, J, P, E
	LOX5	AF039651	9	T
Broad bean (*Vicia faba*)	VfLox1	Z73498		N, R

[a] As archived in GenBank; please consult these accession nos. for more details about the pertinent authors. [b] Activity determined from a cDNA expressed in *Escherichia coli*. [c] Abbreviations: ABA, abscisic acid; BTH, benzo(1,2,3)thiadiazole-7-carbothioic acid; E, elicitor; Et, ethylene; F, fruit; Fl, flower; In, inflorecen; G, germination; INA, 2,6-dichlorolsonicotinic acid; J, jasmonates; L, leaf; N, nodule; P, pathogen; Po, pod; R, root; S, seed; St, stem; Sy, systemin; T, tuber; VSP, vegetative storate protein; W, wounding; Ws, water stress; and S, seed. [d] Chloroplastic. [e] Lipid bodies. [f] Cytosolic. [g] Vacuolar LOX.

to be related to the synthesis of a number of different compounds with signaling activity (Table I; Creelman and Mullet, 1997; Bate and Rothstein, 1998).

The necessity of a chloroplastic isoform of LOX for a normal wound response in Arabidopsis (Atlox2; Bell et al., 1995) and potato (H3; Royo et al., 1999) has been clearly established. There is a reduction of the accumulation of wound-inducible mRNAs in transgenic plants with diminished levels of AtLOX2 and H3, indicating that the synthesis of some oxylipins that function in the wounding response is initiated in the chloroplast. Additional support for this idea is the demonstration that chloroplastic monogalactosyl diacylglycerols decrease after wounding, suggesting that these lipids are the source of linolenic acid for oxylipin synthesis (Conconi et al., 1996), and that the chloroplast envelope membranes contain enzymes that catalyze the synthesis of several oxylipins (Blee and Joyard, 1996). Nevertheless, because nonchloroplastic LOXs are also induced upon wounding (Porta et al., 1999), the synthesis of wound-inducible oxylipins in different cellular compartments exists as well.

There is abundant evidence supporting the role of JA and phytodienoic acid (OPDA) as signaling molecules in the response to wounding. JA and OPDA levels increase upon wounding (Creelman and Mullet, 1997; Parchmann et al., 1997). JA or OPDA treatment induces the synthesis of molecules that function in the defense against herbivores (Creelman and Mullet, 1997). Also, Arabidopsis plants defective in either the synthesis or perception of JA are insensi-

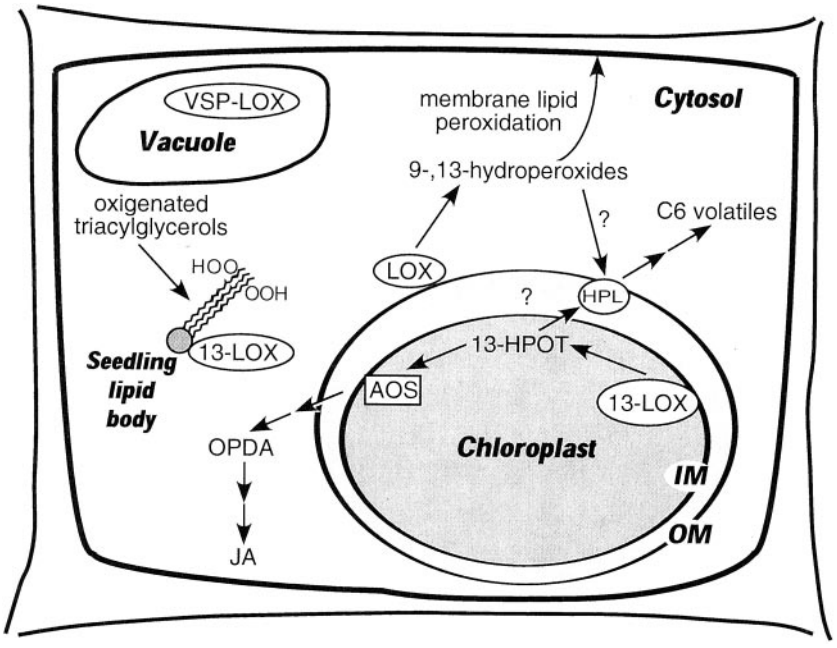

Figure 3. Proposed model of the compartmentalization of LOXs and other enzymes of the oxylipins pathway. Chloroplastic 13-LOX produces 13-HPOT for the synthesis of JA through the action of AOS, which is localized in the inner envelope membrane (IM) of the chloroplast, and probably also for the synthesis of other defense related oxylipins. The 9-,13-hydroperoxides for the synthesis of C6 volatiles via the HPL located in the outer envelope membrane (OM) of the chloroplast are produced by cytosolic or chloroplastic LOXs. The 9-,13-hydroperoxides are also involved in the membrane lipid peroxidation that eventually produces a hypersensitive response (HR) in pathogen-infected plants. During germination of cucumber seeds, 13-LOX adds oxygen to triacylglycerols before the action of lipases and phospholipases. Cytosolic and chloroplastic LOXs may be in the proximity of the membrane. ?, Probable origin of hydroperoxides. For the sake of simplicity, different enzymes are shown in a single cell.

tive to wounding and insect attack responses (McConn et al., 1997; Xie et al., 1998). Similarly, Arabidopsis plants with cosuppressed expression of the nuclear gene coding for the chloroplastic Atlox2 have diminished levels of JA and have reduced expression levels of the wound-induced *vsp* gene (Bell et al., 1995). Wound-induced LOXs are also induced by exogenous JA (Porta et al., 1999), suggesting a feedback mechanism in the synthesis of this growth regulator and a role for this compound in regulating the synthesis of other wound-inducible oxylipins. Although the role of JA in response to wounding has been studied extensively, it is important to emphasize that other oxylipins besides JA are synthesized after wounding in plants, and therefore also play an important role in this response (Table I).

C6-volatiles, aldehydes, and alcohols, all products of the HPL pathway, are synthesized rapidly upon wounding. These compounds also act as signaling molecules in the defense response. In Arabidopsis, (*E*)-2-hexenal induces a subset of JA-induced genes associated with the defense response, but fails to induce some other JA-responsive genes (Bate and Rothstein, 1998). This indicates that different signals from the LOX-derived pathways are mediators in the wounding response. In this respect, it is interesting that leaves of transgenic potato plants with reduced LOX H3 levels produce slightly more JA than wild-type plants in unwounded leaves or soon after wounding. JA treatment of these potato antisense plants does not induce proteinase inhibitor II (PIN2) mRNA at wild-type levels (Royo et al., 1999). Thus, an oxylipin different from JA may be involved in the induction of PIN2 mRNA accumulation after wounding. Recently, it was demonstrated that AOS and HPL are localized in different membranes of the chloroplast envelope (Froehlich et al., 2001). It is possible that distinct LOXs associate with different membranes in the chloroplast, and therefore with enzymes of different pathways. This would lead to the compartmentalization of oxylipin synthesis in the chloroplast (Fig. 3).

When plants are damaged by insects, the amount of volatiles normally released by an intact plant increases significantly. These volatiles are qualitatively and/or quantitatively different from those emitted by undamaged or mechanically wounded plants (Arimura et al., 2000). Some of these compounds, such as (*Z*)-3-hexenyl acetate (Alborn et al., 1997), are products of the LOX pathway. Herbivore-induced volatiles are attractors of natural predators of the attacking herbivores (Agrawal, 2000). The emission of these volatiles is increased by oral secretion products from herbivores. One of these products is volicitin [*N*-(17-hydroxylineloyl)-L-Gln], which is found in the oral secretions of beet armyworm (*Spodoptera exigua*) caterpillars. This compound alone, when applied to maize (*Zea mays*) plants, elicits the release of volatiles that attract parasitic wasps (*Cotesia marginiventris*; Alborn et al., 1997). Because of the structure of volicitin, which is related to products of the AOS pathway, it has been suggested that this compound could be involved in activating LOX-mediated defense responses in plants (Alborn et al., 1997).

Lima bean (*Phaseolus lunatus*) plants infested with spider mites (*Tetranychus urticae*) accumulate transcripts of LOX and five other defense genes together with an increase in LOX activity. When applied to uninfested leaves, the volatiles produced by infested lima bean leaves, mainly terpenoids, elicit a similar increase in LOX activity and mRNA levels. Four of the other defense genes are also induced in these

leaves (Arimura et al., 2000). Pretreatment with salicylhydroxamic acid, an inhibitor of LOX activity, blocks the expression of both LOX and the defense genes. The effect of the inhibitor could be circumvented by the simultaneous application of JA. Hence, the plant response to spider mites seems to be mediated by JA. Because volatiles produced by infested leaves are different from wound-induced volatiles, it has been observed that neighboring plants can differentiate between these stimuli and respond accordingly (Arimura et al., 2000). JA induces a similar, although not identical, blend of volatiles to those produced by herbivores (Agrawal, 2000). Exogenous application of JA to tomato plants in the field causes increased parasitism of beet armyworm larvae by its natural enemy, the wasp *Hyposoter exiguae* (Thaler, 1999). Thus, LOX pathway products function in plant protection against herbivores through the induction of several defense molecules and by attracting herbivore predators (Thaler, 1999).

Pathogen Attack

Induction of LOX genes during plant-pathogen interactions has been reported in several species. As shown in Table I, the function of LOX in the defense against pests seems to be related to the synthesis of a number of different compounds with signaling functions (Creelman and Mullet, 1997; Parchmann et al., 1997), antimicrobial activity (Croft et al., 1993; Weber et al., 1999), or to the development of the HR (Rustérucci et al., 1999).

In tobacco, 9-LOX activity and Lox1 mRNA expression are induced upon infection by *Phytophtora parasitica* var *nicotianae*. Interestingly, both 9-LOX activity and Lox1 mRNA expression appear earlier in an incompatible plant-pathogen interaction than in a compatible one, thus supporting a role for this 9-LOX in plant defense against fungal infection (Rancé et al., 1998). Transgenic tobacco plants for an antisense Lox1 construct, infected with an incompatible race of *P. parasitica* var *nicotianae*, develop disease symptoms similar to those observed in a compatible interaction (Rancé et al., 1998). Two possible explanations exist for this finding: (a) Metabolites from the LOX pathway with antifungal activity are no longer synthesized, thus allowing fungal growth. Both colneleic and colnelenic acids, two LOX-derived compounds with antimicrobial activity, are synthesized upon pathogen infection in the potato-*P. infestans* interaction (Weber et al., 1999); in the *P. vulgaris-Pseudomonas syringae* pv *phaseolicola* interaction, the LOX-derived compound with antimicrobial activity is (*E*)-2-hexenal (Croft et al., 1993); and (b) Some product of LOX metabolism is required to induce the HR, which is a pathogen-induced cell death process at the site of infection in an incompatible interaction that limits pathogen growth. The HR is characterized by the loss of membrane integrity and closely related to the generation of lipid peroxides and active oxygen species. It has been postulated that LOX-mediated lipid oxidation is important in causing membrane damage during the HR. Direct evidence in support of the role of LOX in lipid peroxidation during the HR has been reported (Rustérucci et al., 1999). Tobacco leaves infiltrated with the elicitor-protein cryptogein induce massive chloroplastic lipid peroxidation dependent on 9-LOX metabolism. An increase in 9-LOX activity and tobacco Lox1 mRNA also occurs after cryptogein infiltration. Leaf necrosis correlates with the level of fatty acid peroxidation, and necrosis can be induced by linolenic or linoleic acids in leaves previously treated with methyl jasmonate as an inducer of 9-LOX activity (Rustérucci et al., 1999). It appears that in cryptogein-infiltrated tobacco leaves, the activity of two LOXs is required for the progression of the HR: a 13-LOX for the synthesis of JA and a 9-LOX for fatty acid peroxidation, leading to membrane damage and eventual cell death (Rustérucci et al., 1999; Fig. 3). In lentil root protoplasts, hydrogen peroxide, which is produced in plants early during the HR, induces cell death and an increase in LOX activity. LOX inhibitors and anti-LOX antibodies protect protoplasts against hydrogen peroxide-induced cell death. Moreover, 9- and 13-HPOT (defined in Fig. 1) cause cell death in this system (Maccarrone et al., 2000).

CONCLUDING REMARKS

The study of transgenic lines and of the physiological role of different oxylipins have made clear that LOX is not only important for the synthesis of JA, but also of a number of other products that have specific roles in development and in responses to stress.

In vitro, the preferred substrates for LOXs are free fatty acids. In vivo, however, cucumber lipid body LOX (Feussner et al., 2001) and a chloroplastic Arabidopsis LOX (Stelmach et al., 2001) use sterified fatty acids as substrates. This observation introduces the question of whether there are other LOXs that add oxygen to fatty acids before the action of lipases or phospholipases.

The observation that chloroplast envelope membranes contain enzymes that catalyze the synthesis of several oxylipins (Blee and Joyard, 1996) and that chloroplastic lipids decrease after wounding or pathogen attack (Conconi et al., 1996; Rustérucci et al., 1999) suggest that oxylipin synthesis during such stresses is initiated in the chloroplast. An integrative role for the chloroplast during wounding and pathogen attack needs to be explored.

The analysis of the phenotype of the antisense LOX transgenic potato plants demonstrates that LOX-derived products have a critical function during potato tuber development (Kolomiets et al., 2001). Other developmental programs, such as germination and nodule formation, may require oxylipins as sig-

naling molecules that regulate processes of growth and metabolism.

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