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INVITED REVIEW

Seed lipoxygenases: occurrence and functions

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Abstract

Lipoxygenases are widely distributed in the animal and plant kingdoms. These enzymes catalyse the hydroperoxidation of polyunsaturated fatty acids containing *cis*,*cis*-1,4-pentadiene moieties. Multiple isoenzymes, with different biochemical properties and tissue distribution, have been described for many plants. Lipoxygenases occur in vegetative tissues, but also accumulate in various seeds, and especially in leguminous seeds. Although several functions have been proposed for vegetative lipoxygenases, the roles of seed lipoxygenases remain enigmatic. In this review we discuss whether physiological functions assigned to vegetative lipoxygenases can be extended to seed isoforms.

Keywords: fatty acid hydroperoxide, jasmonate, seed lipoxygenase, storage protein, stress resistance

Introduction

Lipoxygenases (linoleate:oxygen oxidoreductase, EC 1.13.11.12) are a class of non-haem iron-containing dioxygenases that catalyse the oxygenation of polyunsaturated fatty acids with *cis*,*cis*-1,4-pentadiene structures, such as linoleic and linolenic acids, to form conjugated diene hydroperoxides (Fig. 1). The enzymes are widely distributed in both plants and animals (Vick and Zimmerman, 1987; Siedow, 1991; Yamamoto, 1992; Rosahl, 1996; Brash, 1999; Kühn and Thiele, 1999).

Besides their physiological role, plant lipoxygenases are of significant importance to the food industry, since these enzymes have been implicated in the generation of the flavour and aroma in many plant products. For instance, they are responsible for the undesirable 'beany', 'green' and 'grassy' flavours produced during processing and storage of protein products derived from legume seeds (Fukushima, 1994; Robinson *et al*., 1995) and the development of the stale flavour in beer during storage (Kobayashi *et al*., 1993, 1994). Lipoxygenases also play an important role in the baking industry. They are quite effective as bleaching agents, increase mixing tolerance and improve dough rheology (Nicolas and Potus, 1994; Larreta-Garde, 1995; Cumbee *et al*., 1997; Borrelli *et al*., 1999).

A survey of the literature indicates that lipoxygenases are present in most, if not all, plant organs, depending on developmental stage and environment (i.e. after a stress). Early studies reported that most plant lipoxygenases are soluble enzymes located predominantly in the cytosol (Siedow, 1991). However, increasing experimental evidence shows that lipoxygenases can be found in other compartments, as well as in association with microsomal and plasma membranes (Table 1). Charge modifications of the soluble lipoxygenases may permit their association with membranes (Droillard *et al*., 1993), but non-specific adsorption to membrane fractions has also been observed (Siedow and Girvin, 1980; Mack *et al*., 1987).

Several lipoxygenase isoforms have been identified in different plant species. Their biochemical properties, gene structure and expression, developmental regulation, tissue distribution and physiological roles have been studied mainly in soybean (for reviews, see Axelrod, 1974; Gaillard and Chan, 1980; Mack *et al*., 1987; Hildebrand, 1989; Siedow, 1991; Gardner, 1995; Rosahl, 1996; Shibata, 1996; Casey, 1999). The various isoforms have been classified as two types according to two criteria. The first, and older criterion, relies on catalytic behaviour, such as the pH for optimum activity and the positional specificity for the hydroperoxide substrates (Siedow, 1991). Type-1 lipoxygenase [the original enzyme crystallized from soybeans by Theorell *et al*. (1947), later designated lipoxygenase-1 (Christopher *et al*., 1970)] has optimum activity at pH 9–10. Type-2 lipoxygenases generally

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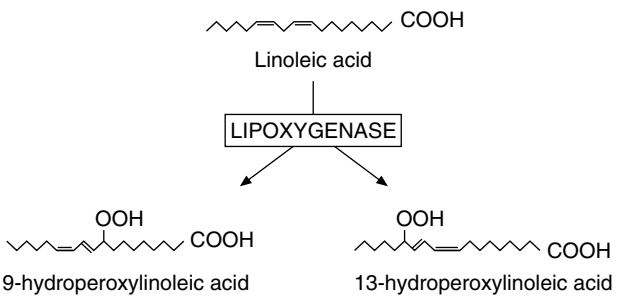

Figure 1. The formation of 9- and 13-hydroperoxides from linoleic acid by lipoxygenases.

have pH optima of 6–7. Some type-2 lipoxygenases also catalyse secondary reactions leading to pigment bleaching and production of oxodienoic acids (Klein *et al*., 1985; Siedow, 1991). Most plant lipoxygenases belong to the type-2 form, soybean lipoxygenase-1 appearing to be an exception (Gaillard and Chan, 1980). A recent alternative classification based on amino-acid sequence similarity (Shibata, 1996) has also been developed. Plant lipoxygenases genes can be divided into two categories based on whether the gene product has a transit peptide (Lox 2) or not (Lox 1). Most plants lipoxygenase genes isolated so far fall into the Lox 1 class, except those from *Arabidopsis thaliana* and rice (Shibata, 1996).

In higher plants, two major pathways involving lipoxygenase have been described for the metabolism of fatty acid hydroperoxides. They are known collectively as the 'lipoxygenase pathway' (Fig. 2). One branch of the lipoxygenase pathway produces traumatic acid, a compound that may be involved in plant cell wound responses (Zimmerman and Coudron, 1979), and volatile C_6-aldehydes and C_6-alcohols. These volatile compounds are the major contributors to the characteristic fresh 'green' odour emitted by leaves (Hatanaka, 1996) and may play a role in pathogen defence (Croft *et al*., 1993). The second branch produces jasmonic acid, a molecule likely to serve a regulatory role in plant cells (Staswick, 1992; Sembdner and Parthier, 1993). Further details about the lipoxygenase pathways can be found in recent reviews (Vick, 1993; Gardner, 1995, 1996; Fauconnier and Marlier, 1997; Grechkin, 1998).

Table 1. Subcellular localization of non-cytosolic lipoxygenases in various species

Subcellular localization	Organ	Species	References
Microsomal membranes	Leaf	*Medicago sativa* L.	Grossman *et al*. (1972)
	Fruit	*Lycopersicon esculentum* L.	Todd *et al*. (1990), Bowsher *et al*. (1992), Droillard *et al*. (1993)
	Fruit	*Fragaria* × *ananassa* Duch.	Pérez *et al*. (1999)
	Stem	*Pisum sativum* L.	Braidot *et al*. (1993)
	Petal	*Dianthus caryophyllus* L.	Rouet-Mayer *et al*. (1992)
	Tuber	*Solanum tuberosum* L.	Bostock *et al*. (1992)
	Cotyledon	*Cucumis sativus* L.	Feussner and Kindl (1994)
Plasmalemma	Fruit	*Lycopersicon esculentum* L.	Droillard *et al*. (1993)
	Hypocotyl	*Glycine max* L.	Macri *et al*. (1994)
Tonoplast	Fruit	*Lycopersicon esculentum* L.	Droillard *et al*. (1993)
Vacuole	Leaf	*Glycine max* L.	Tranbarger *et al*. (1991), Grimes *et al*. (1992), Feussner *et al*. (1995), Klauer *et al*. (1996), Stephenson *et al*. (1998)
	Cotyledon	*Glycine max* L.	Grimes *et al*. (1992)
	Fruit	*Cucumis sativus* L.	Wardale and Lambert (1980)
Mitochondria	Leaf	*Medicago sativa* L.	Grossman *et al*. (1972)
	Stem	*Pisum sativum* L.	Braidot *et al*. (1993)
	Seedling	*Pisum sativum* L.	Haydar and Hadziyev (1973)
Chloroplast	Leaf	*Medicago sativa* L.	Grossman *et al*. (1972)
	Leaf	*Hordeum vulgare* L.	Feussner *et al*. (1995)
	Leaf	*Lycopersicon esculentum* L.	Heitz *et al*. (1997)
	Leaf	*Spinacia oleracea* L.	Blée and Joyard (1996)
	Shoot	*Pisum sativum* L.	Douillard and Bergeron (1981)
Plastid	Hypocotyl	*Glycine max* L.	Grimes *et al*. (1992)
Peroxisome	Seedling	*Pisum sativum* L.	Haydar and Hadziyev (1973)
Nucleus	Leaf	*Glycine max* L.	Feussner *et al*. (1995)
Oil body	Cotyledon	*Cucumis sativus* L.	Feussner and Kindl (1992, 1994)
	Cotyledon	*Helianthus annuus* L.	Rodriguez-Rosales *et al*. (1998)
	Cotyledon	*Pimpinella ansium* L.	Radetzky *et al*. (1993)

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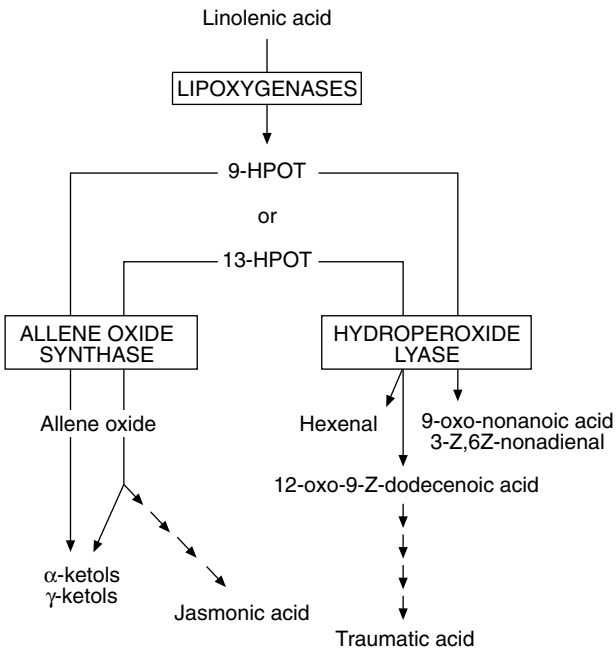

Figure 2. Overview of the lipoxygenase pathway. 9-HPOT, 9(*S*)-hydroperoxy-*trans*-10,*cis*-12,*cis*-15-octadecatrienoic acid; 13-HPOT, 13(*S*)-hydroperoxy-*cis*-9,*trans*-11,*cis*-15-octadecatrienoic acid.

Efforts have been directed at unravelling structure–function relationships of lipoxygenases (Gardner, 1991, 1995, 1996; Siedow, 1991; Vick, 1993; Martini and Iacazio, 1995; Fauconnier and Marlier, 1997; Grechkin, 1998; Casey, 1999; Grechkin and Tarchevsky, 1999). However, the physiological functions of plants lipoxygenases are not yet clearly understood, due to the presence of many isoenzymes and the diversity of the end-products of the lipoxygenase pathway. Lipoxygenases have been implicated in numerous physiological processes such as growth and development (Kubacka-Zebalska and Kacperska-Palacz, 1980; Mauch *et al*., 1997), senescence (Leshem, 1988; Yao *et al*., 1993; van Leyen *et al*., 1998), plant response to pathogens (Slusarenko, 1996), wounding (Creelman *et al*., 1992b; Farmer and Ryan, 1992) and abiotic stress (Maccarrone *et al*., 1991, 1992; Todd *et al*., 1992). Some lipoxygenase isoenzymes may also function as vegetative storage proteins (Tranbarger *et al*., 1991; Grimes *et al*., 1993; Kato *et al*., 1993). Several hypotheses have been put forward explaining the multiple physiological roles of lipoxygenases. These enzymes are supposed to be active via their involvement in the biosynthesis of several growth regulators, such as abscisic acid, traumatin and jasmonates (Zimmerman and Coudron, 1979; Vick and Zimmerman, 1983; Creelman *et al*., 1992a; Mueller, 1997; Sheng *et al*., 2000). Moreover, intermediates of the lipoxygenase pathway can be involved in intracellular signalling (Karimova *et al*., 1999; Sheng *et al*., 2000). Lipoxygenases may also contribute to cell elongation or degradation by modifying cell membrane phospholipid composition. Although these enzymes are thought to act on free fatty acids, tomato pericarp lipoxygenase can catalyse the specific oxygenation of esterified fatty acids of membrane phospholipids (Droillard *et al*., 1993).

Most of these lipoxygenase functions have been suggested for enzymes located in vegetative organs. However, while legume seeds are characteristically well-endowed with large amounts of lipoxygenases, their physiological roles in seeds remain enigmatic. The present paper focuses on lipoxygenase occurrence in seeds and examines whether the putative roles of lipoxygenases in vegetative tissues are similar to those of lipoxygenases in developing seeds.

Lipoxygenase occurrence in seeds

Seed lipoxygenases were first thought to be restricted to legumes and certain cereals. It is now clear that lipoxygenases are present in seeds of many species (Table 2), and it is tempting to postulate that they occur in most seeds. Examples of seed extracts lacking apparent *in vitro* activity may simply be due to the lack of sensitivity of the classical assays employed (Axelrod, 1974). Moreover, endogenous lipoxygenase inhibitors such as phenolic compounds (Richard-Forget *et al*., 1995; Kohyama *et al*., 1997; Kubicka *et al*., 1999) may have complicated the detection of lipoxygenase in plant extracts. This might be the reason why lipoxygenase activity has been detected in seeds of *Zea mays*, *Helianthus annuus* and *Brassica napus* by some authors and not by others (Table 2). As suggested by Siedow (1991), the development of very sensitive assays using Northern blotting, Western immunoblots and ELISA techniques should leave no question about the presence or absence of lipoxygenase transcripts and proteins in a given tissue at a particular stage of development. To date, the different isoforms have been characterized mainly in legume and cereal seeds where they are particularly abundant.

Legume seeds

Chang and McCurdy (1985) grouped 14 legumes into three classes based on their lipoxygenase specific activity *in vitro*. Legumes with a high level of activity were soybean, *Vigna unguiculata* and *Lens culinaris*; legumes that possessed a medium level of activity

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Table 2. Occurrence of seed lipoxygenases

Presence of seed lipoxygenase		Absence of seed lipoxgenase	
Species	References	Species	References
Arachis hypogaea	Chiou *et al*. (1997)	*Arabidopsis thaliana*	Melan *et al*. (1994)
Brassica napus	Meshehdani *et al*. (1990)	*Beta vulgaris*	Loiseau, unpublished results
Cajanus cajan	Kalpana and Rao (1993)	*Brassica napus*	Fauconnier *et al*. (1995)
Cicer arietinum	Sanz *et al*. (1992)	*Citrullus lanatus*	Vick and Zimmerman (1976)
Glycine max	Theorell *et al*. (1947)	*Cucumis sativus*	Matsui *et al*. (1992)
Helianthus annuus	Kubicka *et al*. (1999)	*Dactylis glomerata*	Fauconnier *et al*. (1995)
Hordeum vulgare	Doderer *et al*. (1992)	*Fagopyrum esculentum*	Loiseau, unpublished results
Lens culinaris	Chang and McCurdy (1985)	*Festuca pratensis*	Fauconnier *et al*. (1995)
Linum usitatissimum	Oomah *et al*. (1997)	*Gossypium hirsutum*	Vick and Zimmerman (1981)
Lolium perenne	Fauconnier *et al*. (1995)	*Helianthus annuus*	Fauconnier *et al*. (1995)
Lotus corniculatus	Fauconnier *et al*. (1995)	*Lolium multiflorum*	Fauconnier *et al*. (1995)
Lupinus albus	Najid *et al*. (1988)	*Lolium perenne*	Fauconnier *et al*. (1995)
Medicago sativa	Fauconnier *et al*. (1995)	*Phleum pratense*	Fauconnier *et al*. (1995)
Oryza sativa	Ida *et al*. (1983)	*Poa trivialis*	Fauconnier *et al*. (1995)
Phaseolus angularis	Chang and McCurdy (1985)	*Raphanus sativus*	Fauconnier *et al*. (1995)
Phaseolus aureus	Chang and McCurdy (1985)	*Zea mays*	Fauconnier *et al*. (1995)
Phaseolus lunatus	Chang and McCurdy (1985)		
Phaseolus vulgaris	Eiben and Slusarenko (1994)		
Pisum sativum	Eriksson and Svensson (1970)		
Prunus dulcis	Zacheo *et al*. (1998)		
Trifolium arvense	Fauconnier *et al*. (1995)		
Trifolium pratense	Fauconnier *et al*. (1995)		
Triticum aestivum	Hertel *et al*. (1987)		
Triticum durum	Manna *et al*. (1998)		
Vicia faba	Fauconnier *et al*. (1995)		
Vicia sativa	Fauconnier *et al*. (1995)		
Vigna unguiculata	Chang and McCurdy (1985)		
Zea mays	Belefant and Fong (1991)		

were *Phaseolus angularis*, *Vicia faba*, *Pisum sativum* and five biotypes of *Phaseolus vulgaris* (black bean, great Northern bean, navy bean, Pinto bean, red kidney bean). Legumes having a low level of activity were *Cicer arietinum*, *Phaseolus lunatus* and *Phaseolus aureus*. Specific activities of lentil and cowpea lipoxygenases were higher than those of soybean at pH 6.9.

Soybean seed lipoxygenases are abundant enzymes that constitute 1–2% of the total protein content. They exist in three isoenzymatic forms, the properties of which are summarized in Table 3. Soybean seed isoenzymes are 94–97 kDa monomeric proteins with distinct isoelectric points ranging from about 5.7 to 6.4, and can be distinguished by pH optimum, substrate specificity, product formation and stability (see Mack *et al*., 1987; Siedow, 1991 for reviews). LOX-1 is the smallest in size (838 amino acids; 94 kDa), exhibits maximal activity at pH 9.0 and converts linoleic acid preferentially into the 13-hydroperoxide derivative. LOX-2 is characterized by a larger size (865 amino acids; 97 kDa), by a peak of activity at pH 6.8, and forms equal amounts of the 13- and 9-hydroperoxide compounds. Vernooy-Gerritsen *et al*. (1984) have reported a pH 9.0 optimum for both LOX-1 and LOX-2 isoenzymes *in vivo*. LOX-2 oxygenates the esterified unsaturated fatty acid moieties in membranes, in contrast to LOX-1, which only uses free fatty acids as substrates (Maccarrone *et al*., 1994). LOX-3 (857 amino acids; 96.5 kDa) exhibits its maximal activity over a broad pH range centred around pH 7.0 and displays a moderate preference for producing a 9-hydroperoxide product. It is the most active isoenzyme with respect to both carotenoid co-oxidation and production of oxodienoic acids (Ramadoss *et al*., 1978).

Pea seed lipoxygenases have been purified in several laboratories. However, there are conflicting reports as to isoenzyme number and molecular masses (Eriksson and Svensson, 1970; Arens *et al*., 1973; Haydar and Hadziyev, 1973; Haydar *et al*., 1975; Yoon and Klein, 1979; Reynolds and Klein, 1982; Chen and Whitaker, 1986; Sanz *et al*., 1993). Pea seed lipoxygenases are characterized by two major isoforms of 95 kDa, equivalent to soybean LOX-2 and LOX-3 (Table 3), and two less abundant isoenzymes, one of which corresponds to soybean LOX-1 (Yoon and Klein, 1979; Reynolds and Klein, 1982; Domoney *et al*., 1990; Guerdam *et al*., 1993). As in soybean, LOX-1, −2 and −3 in pea differ in their product specificity (Sanz *et al*., 1993; Wu *et al*., 1995; Hughes *et al*., 1998)

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Table 3. Some biochemical properties of the major lipoxygenase isoforms found in legume and cereal seeds

Species	Name	pI	MW (kDa)	Optimum pH	Product specificity [b]
Soybean	LOX-1	5.68; 5.96 [a]	96; 94.37 [a]	9.0	13-HPOD
	LOX-2	6.25; 6.27 [a]	96; 97.14 [a]	6.8	13-HPOD = 9-HPOD
	LOX-3	6.15; 6.26 [a]	96; 96.76 [a]	7.0	13-HPOD < 9-HPOD
Pea	LOX-2	6.06 [a]	94; 97.13 [a]	5.8–6.4	13-HPOD > 9-HPOD
	LOX-3	6.07 [a]	97; 97.63 [a]	5.6–6.5	13-HPOD < 9-HPOD
Chickpea	CL-1	nd	97	6.0	13-HPOD < 9-HPOD
	CL-2	nd	97	5.5	13-HPOD
Kidney bean	LOX-1	nd	nd	5.7	13-HPOD = 9-HPOD
	LOX-2	nd	nd	5.7	13-HPOD
Broad bean	BBL-1	nd	97	5.8	13-HPOD = 9-HPOD
	BBL-2	nd	97	5.8	13-HPOD
Lentil	C1	5.4–5.5	94	6.5–9	13-HPOD < 9-HPOD
	C2	5.2–5.3	94	6.5	13-HPOD
Lupin	L-1	5.35	71	6.0	13-HPOD
Barley	LOX-1	5.2; 5.63[a]	90; 96.39[a]	6.5	9-HPOD
Maize	LOX-1	6.4	100	7	13-HPOD
	LOX-2	5.5–5.7	90	6–9	9-HPOD
Rice	LOX-1	nd	nd	4.5	13-HPOD = 9-HPOD
	LOX-2	5.86[a]	96.66[a]	5.5	13-HPOD = 9-HPOD
	LOX-3	nd	93	7.0	9-HPOD
Wheat	L-1	nd	110	5.5	nd
	L-2	nd	110	5.5	nd
	L-3	nd	110	4.5–6.0	nd

[a] Calculated from amino-acid sequences (available at Swiss-Prot database), pI was calculated according to Bjellqvist *et al.* (1993).
[b] From linoleic acid as substrate. 13-HPOD, 13-hydroperoxylinoleic acid; 9-HPOD, 9-hydroperoxylinoleic acid.
nd = not determined.

and their ability to oxidize esterified linoleic acid (Hughes *et al.*, 1998). Only LOX-3 is effective in chlorophyll bleaching and carbonyl production (Yoon and Klein, 1979; Hughes *et al.*, 1998).

Seeds of chickpea, lentil, broad bean and kidney bean contain two major lipoxygenases, one synthesizing mainly 13-hydroperoxide from linoleic acid, whereas the other produces 9- and the 13-hydroperoxides and 9- and 13-ketodienes (Sanz *et al.*, 1993; Hilbers *et al.*, 1995; Clemente *et al.*, 2000). The lupin lipoxygenase shows maximum activity at pH 6.0 and forms 13-hydroperoxide from linoleic acid (Najid *et al.*, 1988). Peanut seeds contain three lipoxygenase isoenzymes with biochemical properties similar to the three soybean isoforms (references in Burow *et al.*, 2000).

Cereal seeds

The study of barley lipoxygenase has led to the identification and characterization of only one isoenzyme (Table 3) in dry caryopses (Doderer *et al.*, 1992; Yang *et al.*, 1993; Hugues *et al.*, 1994). This lipoxygenase is localized exclusively in the germ (Yang *et al.*, 1993). It has a molecular mass of approximately 90 kDa and an isoelectric point of almost 5.2 (Doderer *et al.*, 1992; Yang *et al.*, 1993). Its activity has a optimum pH of 6.5 and yields predominately 9-hydroperoxides (Doderer *et al.*, 1992; Yang *et al.*, 1993; Hugues *et al.*, 1994).

In wheat, three major lipoxygenase isozymes (L-1, L-2 and L-3) and one minor isozyme (L-a) have been purified from defatted germ extracts (Shiiba *et al.*, 1991). The molecular masses of the lipoxygenase isoenzymes are approximately 110 kDa. The optimum pH of L-1 and L-2 isozymes is 5.5. L-3 isozyme shows higher activity over a wider pH range, with an optimum pH between 4.5 and 6.0. In *Triticum durum*, three lipoxygenase isoenzymes, L-1, L-2 and L-3 have also been isolated (Hsieh and McDonald, 1984) and studied at the biochemical level.

Three isozymes, LOX-1, LOX-2 and LOX-3, were found in *Oryza sativa* embryos, LOX-3 being the most abundant (Ida *et al.*, 1983). However, a Thai variety, Daw Dam, lacks the LOX-3 protein in dry caryopses (Suzuki *et al.*, 1993). Rice grain lipoxygenase is localized in the bran fraction, but has been not detected in the hull or endosperm fractions (Suzuki and Matsukura, 1997).

In maize, two lipoxygenase isoenzymes, LOX-1 and LOX-2, have been isolated from dry and germinating embryos (Poca *et al.*, 1990; Jensen *et al.*, 1997). The LOX-1 isoenzyme has a molecular mass of 100 kDa, optimal activity at pH 7.0, and catalyses the formation of 13-hydroperoxides. LOX-2 has a molecular mass of 90 kDa, is active in a pH range

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from 6.0 to 9.0, and catalyses the formation of 9-hydroperoxides. The LOX-2 gene is highly expressed during early embryogenesis, whereas LOX-1 transcripts are detectable only in dry embryos. Belefant and Fong (1991) suggested that at least two other lipoxygenase isoforms exist in the endosperm. The total and specific activities of lipoxygenase are generally higher in the embryo than endosperm tissues throughout kernel development.

Why are lipoxygenases stored in some seeds?

The expression of the lipoxygenases in developing seeds appears similar to that of storage proteins in legumes such as soybean (Hildebrand *et al*., 1991) and pea (Loiseau, unpublished results). In addition, optimum soybean seed quality (germination and vigour) and maximum lipoxygenase and C6-aldehyde formation are correlated (Trawatha *et al*., 1993). It was concluded that lipoxygenases might play a physiological role either during seed maturation or during germination and seedling growth. However, identifying specific roles of seed lipoxygenases during germination and seedling growth is complicated by the appearance of vegetative isoforms in germinated seeds. While LOX-1, LOX-2 and LOX-3 activities decrease during the early stages of soybean seedling growth (Peterman and Siedow, 1985; Kato *et al*., 1992), three other isoforms (referred as LOX-4, LOX-5 and LOX-6) are produced in cotyledons (Kato *et al*., 1992); LOX-4 and LOX-6 are also synthesized in growing radicles and hypocotyls (Park and Polacco, 1989; Park *et al*., 1994). New lipoxygenases are induced during the early stages of seedling growth in other species, such as pea (Anstis and Friend, 1974; Chateigner *et al*., 1999), French bean (Eiben and Slusarenko, 1994), lupin (Beneytout *et al*., 1988), lentil (Hilbers *et al*., 1995), barley (Yang *et al*., 1993; Holtman *et al*., 1996), rice (Suzuki and Matsukura, 1997), cucumber (Matsui *et al*., 1992), rape (Kubacka-Zebalska and Kacperska-Palacz, 1980), *Pimpinella ansium* (Radetzky *et al*., 1993), cotton (Vick and Zimmerman, 1981), *Arabidopsis thaliana* (Melan *et al*., 1994), *Papaver somniferum* (Bezakova *et al*., 1994) and watermelon (Vick and Zimmerman, 1976). In contrast with legume seeds, lipoxygenase isoform activities in dry cereal seeds increase during seedling growth (Ohta *et al*., 1986; Yang *et al*., 1993; Jensen *et al*., 1997; Suzuki and Matsukura, 1997). The appearance of new lipoxygenases during germination and seedling growth in legumes, as well as in species devoid of seed lipoxygenases, suggests that seed isoforms and newly synthesized vegetative isoenzymes play different roles.

Based upon the literature on vegetative lipoxygenases (Hildebrand, 1989; Siedow, 1991; Rosahl, 1996), we can tentatively suggest several putative roles for lipoxygenases in seeds: fatty acid peroxidation in membranes or storage lipids, production of growth regulators (jasmonates, abscisic acid), responses to pathogens and nitrogen storage.

Fatty acid peroxidation

Membrane damage that occurs during seed storage contributes to a loss of viability and vigour. Oxidative changes in membrane polyunsaturated fatty acids have been widely invoked to explain the deterioration of stored seeds (Wilson and McDonald, 1986). However, membrane lipids are susceptible to both enzymatic and non-enzymatic peroxidation (Shewfelt and Purvis, 1995), and contradictory results have been reported concerning the involvement of lipoxygenases in lipid peroxidation during seed ageing. Increased activity of lipoxygenase was observed during ageing of almond seeds at 80% relative humidity and 20°C (Zacheo *et al*., 1998). In contrast, accelerated ageing (100% relative humidity and 40°C) of pigeonpea seeds was accompanied by a decrease in lipoxygenase activity (Kalpana and Rao, 1993). Salama and Pearce (1993) found that the amount of conjugated dienes formed during seed ageing was about 17 times greater in onion than cucumber, although lipoxygenase activity was similar. They proposed that the low water content and the high ion concentration of dry seeds could easily affect not only the activity of the enzyme, but also the accessibility of the substrate and the distribution of the enzyme between the membrane and the cytosol. Recent studies of a rice mutant deficient in LOX-3, which is the predominant grain isoform, showed that peroxidation products of unsaturated fatty acids are lower in the mutant. In addition, volatile compounds, derived from the hydroperoxides generated during seed ageing, accumulated to a lesser extent in the *lox-3* mutant compared to the wild type (Suzuki *et al*., 1996, 1999). However, the authors did not establish whether mutant seeds aged faster than the wild type. Therefore, the role of lipoxygenase in seed deterioration remains uncertain.

Some vegetative lipoxygenases in oilseeds are localized in oil-bodies and are thought to be involved in triglyceride mobilization (Feussner and Kindl, 1992; Radetzky *et al*., 1993; Rodriguez-Rosales *et al*., 1998). In contrast, neither seed lipoxygenases nor vegetative isoforms contribute to the storage lipid catabolism in soybean (Wang *et al*., 1999).

A plasma membrane-bound lipoxygenase from soybean cotyledons of 11-day-old seedlings is very similar to the seed LOX-1 isoform (Fornaroli *et al*., 1999). Therefore, it was suggested that soluble enzymes might be transferred, by vesicles, to membranes where they may more easily attack polyunsaturated fatty acids linked to phospholipids or liberated by membrane-bound phospholipases.

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The same fate could be envisaged for type-2 enzymes that are known to be able to oxygenate membranes (Maccarrone *et al*., 1994). Seed lipoxygenases might then contribute significantly to cotyledon senescence accompanying reserve resorption.

Synthesis of growth regulators

Several lines of evidence suggest that seed lipoxygenases may participate in growth hormone synthesis that mediates seed development. Lipoxygenases mediate an essential step in jasmonate synthesis by converting α-linolenic acid to 13-hydroperoxy-linolenic acid (Crozier *et al*., 2000). Many of the enzymes involved in jasmonate biosynthesis, such as allene oxide synthase, have been localized within the chloroplast (Mueller, 1997), and the lipoxygenases involved in jasmonate production are also assumed to be plastidial (Crozier *et al*., 2000). However, Bell *et al*. (1995) presented evidence that the *A. thaliana* plastid lipoxygenase (LOX2) is not essential for maintaining jasmonate levels under normal conditions of growth, but is required for wound-induced synthesis of jasmonates in leaves. In addition, the other lipoxygenase, LOX1, which may be involved in jasmonate synthesis, has no apparent targeting signal. These observations suggest that cytosolic lipoxygenases, such as those found in seeds, could participate in jasmonate production. However, it should be mentioned that most cereal seeds contain lipoxygenase isoforms producing only 9-hydro-peroxides, which are unlikely to drive jasmonate synthesis (Gardner, 1988).

Jasmonate content is generally low in seeds, but increases upon germination (Lopez *et al*., 1987; Creelman and Mullet, 1997). Thus, it remains to be established whether developing seeds are equipped with the complete enzymic machinery for jasmonate synthesis. The activities of some enzymes involved in the jasmonate pathway have been detected in corn and flax seeds (Koda, 1992; Mueller, 1997). Production of jasmonate was also monitored during stratification of apple seeds (Ranjan *et al*., 1994). These experiments suggest that jasmonate can be synthesized in developing seeds. Jasmonates may elicit cell expansion in cotyledons during seed maturation, as suggested for the tuberization process (Koda, 1997).

Exogenously applied jasmonates have contrasting effects on seed germination. They inhibit germination of non-dormant seeds (Yamane *et al*., 1981; Corbineau *et al*., 1988; Wilen *et al*., 1991; Kepczynski and Bialecka, 1997; Nojavan-Asghari and Ishizawa, 1998), but jasmonates break the seed dormancy of *Malus domestica* (Ranjan and Lewak, 1992, 1995). Some of the physiological effects of jasmonates, exogenously applied to plants, seem to be similar to the activities of abscisic acid (for reviews, see Koda, 1992; Sembdner and Parthier, 1993). Jasmonates may mediate water stress reactions (Reinbothe *et al*., 1992b) that occur during physiological dehydration and seed maturation. Some jasmonate-induced proteins in cotton cotyledons exhibit homologies with late embryogenesis abundant proteins (Reinbothe *et al*., 1992a).

Lipoxygenases may be involved in an alternative pathway for abscisic acid (ABA) biosynthesis from the carotenoid, violaxanthin (Belefant and Fong, 1991; Creelman *et al*., 1992a). The role of abscisic acid in desiccation tolerance and inhibition of precocious germination is well known (Bewley and Black, 1994). Whether or not lipoxygenases have a physiological role in seed development by controlling abscisic acid levels requires further studies to confirm that the alternative ABA biosynthetic pathway actually exists.

Responses to pathogen attack

Lipoxygenases have been hypothesized to play a role in the response to plant pathogens (Slusarenko, 1996; Crozier *et al*., 2000). Several lines of evidence suggest a similar role in seeds. Peanut lipoxygenase is induced by *Aspergillus parasiticus* infections of mature cotyledons (Burow *et al*., 2000). Also, the production of several antimicrobial substances proceeds via the lipoxygenase pathway (Doehlert *et al*., 1993; Burow *et al*., 1997). Gardner *et al*. (1990) suggested that the penetration of fungal hyphae causes the release of free fatty acids that are converted to hexanal, which inhibits fungal growth. In another way, lipoxygenases may directly inhibit the lipase secreted by fungi (Satouchi *et al*., 1998). Lipoxygenases may also affect protease inhibitor concentrations in soybean seeds, possibly via the synthesis of jasmonates, which activate protease inhibitor genes (de Carvalho *et al*., 1999). Soybean mutants lacking all seed lipoxygenases may provide an excellent tool for studying the possible roles of lipoxygenases in pathogen defences. Such a soybean mutant contained less protease inhibitor than the wild-type seeds, but unfortunately, the susceptibility of the soybean LOX-less seeds to pathogen attack has not yet been investigated (de Carvalho *et al*., 1999).

The developing embryo is not the first point of contact between reproductive structures and pathogens. The initial barriers, pod walls and seed coats, also contain lipoxygenases able to initiate a cascade of responses to pathogen infection (Dubbs and Grimes, 2000). The role of seed lipoxygenases in resistance against infection remains to be fully characterized.

Seed lipoxygenases as storage proteins

Immunocytochemical studies of soybean seedling cotyledons showed that lipoxygenases are localized

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exclusively within the cytosol of parenchyma cells and in the cytosol and vacuoles of epidermal cells (Vernooy-Gerritsen *et al*., 1984; Wang *et al*., 1999). As seedling growth proceeds, lipoxygenases become confined to cells surrounding the vascular bundle, to the epidermis and to the hypodermis. LOXs are in an aberrant type of protein body in cells surrounding the vascular bundle, whereas in epidermis and hypodermis, they occur in regions in the cytoplasm where vacuoles are about to be formed (Vernooy-Gerritsen *et al*., 1984). This tissue and subcellular localization of lipoxygenases in cotyledons following germination is similar to that observed for vegetative lipoxygenases in soybean leaves (Stephenson *et al*., 1998). In addition, no differences were found between the seed protein contents of soybean lines lacking seed lipoxygenases and normal lines (Pfeiffer *et al*., 1992; Narvel *et al*., 1998), suggesting that the triple-null lines compensated for the loss of the lipoxygenase isoenzymes by increasing the biosynthesis of other seed proteins. With respect to the possible involvement of lipoxygenase in jasmonate synthesis, it is interesting to note that this growth hormone accumulates in sink tissues and regulates accumulation of vegetative storage proteins during seed development of *Arabidopsis* (Crozier *et al*., 2000). Thus, seed lipoxygenases might function as storage proteins to fuel the protein synthesis following germination. The fact that the lipoxygenases are soluble in the cytosol and not embedded in protein bodies makes them more accessible to proteases and, consequently, their amino acids could be rapidly available.

Conclusions

The numerous putative roles assigned to plant lipoxygenases over the past 50 years stem from the existence of multiple isoforms. These enzymes are supposed to have biological effects either via their ability to modulate the physico-chemical properties of cell membranes or by producing regulatory molecules via the lipoxygenase pathway. Their functions in seed maturation remain obscure because of the lack of data concerning their enzymatic activity *in vivo*. Studies on 13- and 9-hydroperoxide ratios and jasmonate content in developing seeds should be useful in this respect. Seed lipoxygenases might serve a dual function as storage proteins and protectants against pathogen attack during germination and early seedling growth. They may also contribute to cotyledon senescence. However, further efforts are necessary to determine the fate of seed lipoxygenase isoforms following germination and seedling establishment: Are they hydrolysed to fuel *de novo* protein synthesis or do they become associated with cell membranes or storage lipids to play a specific function during early seedling growth?

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