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# Formation of oxylipins by CYP74 enzymes

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**Abstract** Lipid peroxidation is common to all biological systems, both appearing in developmentally and environmentally regulated processes. Products are hydroperoxy polyunsaturated fatty acids and metabolites derived there from collectively named oxylipins. They may either originate from chemical oxidation or are synthesized by the action of various enzymes, such as lipoxygenases. Cloning of many lipoxygenases and other key enzymes metabolizing oxylipins revealed new insights on oxylipin functions, new reactions and the first hints on enzyme mechanisms. These aspects are reviewed with respect to metabolism of fatty acid hydroperoxides by an atypical P450 subfamily: the CYP74. Up to now this protein family contains three different enzyme activities: (i) allene oxide synthase leading to the formation of unstable allene oxides which react to ketol and cyclopentenone fatty acids, (ii) hydroperoxide lyase producing hemiacetals decomposing to aldehydes and $\omega$-oxo fatty acids and (iii) divinyl ether synthase which forms divinyl ethers. Signalling compounds such as jasmonates, antimicrobial and antifungal compounds such as leaf aldehydes or divinyl ethers, and a plant-specific blend of volatiles including leaf alcohols are among their numerous products.

**Keywords** Allene oxide synthase · Divinyl ether synthase · Fatty acid hydroperoxides · Hydroperoxide lyase · Lipoxygenase

**Abbreviations**

| | |
|---|---|
| AOC | Allene oxide cyclase |
| AOS | Allene oxide synthase |
| DES | Divinyl ether synthase |
| 9-HPOTE | (9*S*,10*E*,12*Z*,15*Z*)-9-Hydroperoxy-10,12,15-octadecatrienoic acid |
| 13-HPOTE | (9*Z*,11*E*,13*S*,15*Z*)-13-Hydroperoxy-9,11,15-octadecatrienoic acid |
| HPO(D/T)E | Hydroperoxy linole(n)ic acid |
| HPL | Hydroperoxide lyase |
| JA | Jasmonic acid |
| KO(D/T)E | Keto linole(n)ic acid |
| LA | Linoleic acid |
| LeA | Linolenic acid |
| LOX | Lipoxygenase |
| OPDA | (9*S*,13*S*)-12-Oxo phytodienoic acid |
| PUFA | Polyunsaturated fatty acids |

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## Introduction

Oxylipin is a collective term for oxygenated metabolites derived from polyunsaturated fatty acids (PUFAs). Many of them are bioactive compounds involved in signal and defence reactions in mammals, higher plants and algae (Blée 2002; Pohnert 2005). The initial formation of lipid hydroperoxides may either occur by autoxidation or by the action of enzymes, such as lipoxygenase (LOX), α-dioxygenase or by membrane bound cytochrome P450 containing monooxygenases (Brash 1999). In plants the metabolism of PUFAs via a LOX-catalysed step and the subsequent reactions are collectively named LOX pathway (Fig. 1) (Feussner and Wasternack 2002; Gardner 1996). The reaction steps metabolizing LOX-derived fatty acid hydroperoxides are a branching point in this metabolic pathway and till now up to six different secondary enzymatic reactions have been described for plants. All of these metabolic routes are named after the first enzymatic reaction step. Among them the allene oxide synthase (AOS), fatty acid hydroperoxide lyase (HPL) and divinyl ether synthase (DES) metabolic routes have been well characterized so far (Blée 1998; Howe and Schilmiller 2002). Recent cloning of a large number of cDNAs coding for enzymes of the three pathways revealed that all of them belong to one cytochrome P450-containing enzyme subfamily, named CYP74. In contrast to common P450 monooxygenases they do not require molecular oxygen nor NAD(P)H-dependent cytochrome P450-reductase (Noordermeer et al. 2001). The new carbon–oxygen bonds are formed by using an acyl hydroperoxide both as the substrate and the oxygen donor. Another common feature of the CYP74s, which differentiate this subfamily from the others is their low affinity to CO (Matsui 1998).

AOS, referred to as hydroperoxide isomerase or hydroperoxide dehydratase in earlier literature (Gardner 1991), forms unstable allene oxides (Fig. 2), which are hydrolysed into α- and γ-ketol

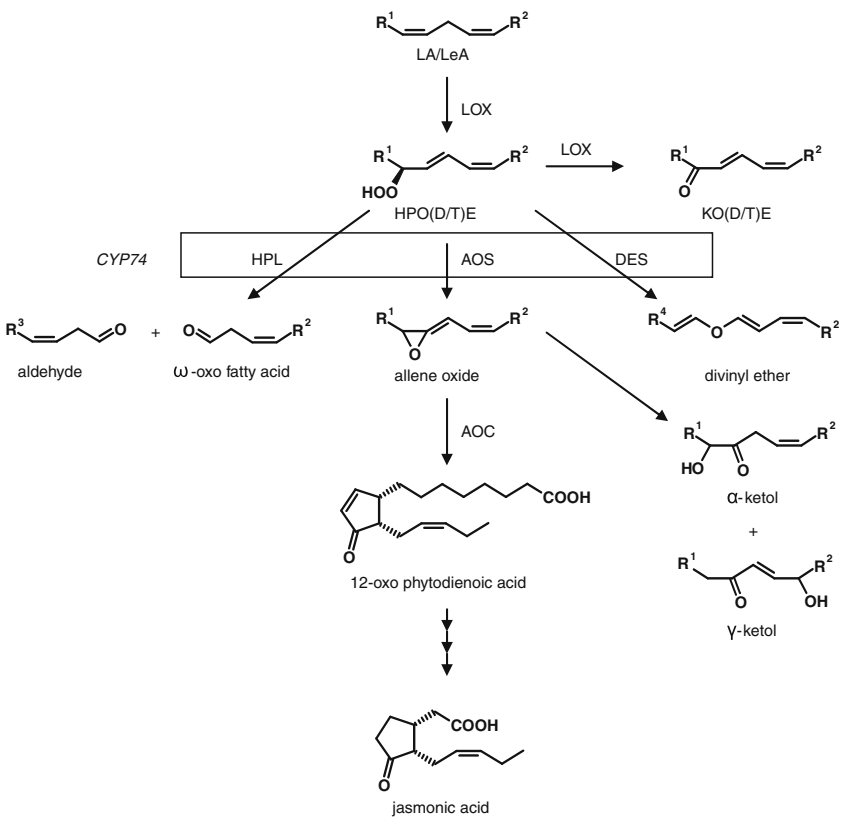


**Fig. 1** Metabolism of PUFAs leading to LOX-derived hydroperoxy PUFAs in plants—The LOX pathway. AOC: Allene oxide cyclase, AOS: Allene oxide synthase, DES: Divinyl ether synthase, HPL: Hydroperoxide lyase, HPO(D/T)E: Hydroperoxy linole(n)ic acid, KO(D/T)E: Keto linole(n)ic acid, LA: Linoleic acid, LeA: Linolenic acid, LOX: Lipoxygenase

fatty acids or can undergo non-enzymatic cyclization. The allene oxide derived from (9*Z*,11*E*,13*S*,15*Z*)-13-hydroperoxy-9,11,15-octadecatrienoic acid (13-HPOTE) may be further converted to a chiral cyclopentanone derivative (9*S*,13*S*)-12-oxo phytodienoic acid (OPDA) by an allene oxide cyclase (AOC). Both enzymes have been characterized as recombinant proteins from several sources (Wasternack and Hause 2002). In addition OPDA acid is converted to jasmonic acid (JA) by several enzymatic steps.

HPL catalyses the oxidative cleavage of the hydrocarbon backbone of fatty acid hydroperoxides. This leads to the formation of short chain aldehydes and the corresponding $\omega$-oxo fatty acids (Fig. 3). As for AOS recombinant enzymes are available from numerous sources (Noordermeer et al. 2001).

Within the DES pathway divinyl ether fatty acids are formed (Fig. 4). Up to now this activity is only known from a few plant species.

## Substrate specificity of CYP74s

Because of the regiospecificity of LOX, (9*S*)- and (13*S*)-hydroperoxides of linoleic and linolenic acids are formed which serve as substrate for the downstream enzymes in plants. Therefore CYP74 can be distinguished into 9- or 13-hydroperoxide specific and unspecific enzymes. The AOS from flax was the first member of the CYP74 family to be cloned (Song et al. 1993b). Therefore, AOS are grouped as CYP74A (Fig. 5). Within this subfamily all enzymes are specific for (13*S*)-hydroperoxides as substrates and thus are named 13-AOS. In contrast, enzymes from barley and rice show no substrate specificity for either (9*S*)-hydroperoxides or (13*S*)-hydroperoxides, respectively, and are therefore called 9/13-AOS. The first member of this group was isolated from barley leaves (Maucher et al. 2000). cDNAs coding for 9-AOS have been isolated from tomato and potato (Itoh et al. 2002; Stumpe et al. 2006b). 9/13- as well as 9-AOS have been grouped into the subfamily CYP74C due to their sequence similarity against each other. This subfamily contains also HPLs accepting either (9*S*)- or (13*S*)-hydroperoxides as substrates (Matsui et al. 2000b) and a specific 9-HPL recently characterized from almond (Mita et al. 2005).

HPL having a preference for (13*S*)-hydroperoxides form the CYP74B subfamily. For the 13-HPLs from *A. thaliana* and tomato it has been shown that they also accept hydroperoxides derived from C20 PUFAs and act with the (9*S*)-hydroperoxide from $\gamma$-linolenic acid as 9-HPLs (Kandzia et al. 2003; Matsui et al. 2000a). Although the enzymes (AOS and HPL) from the moss *Physcomitrella patens* do not group into

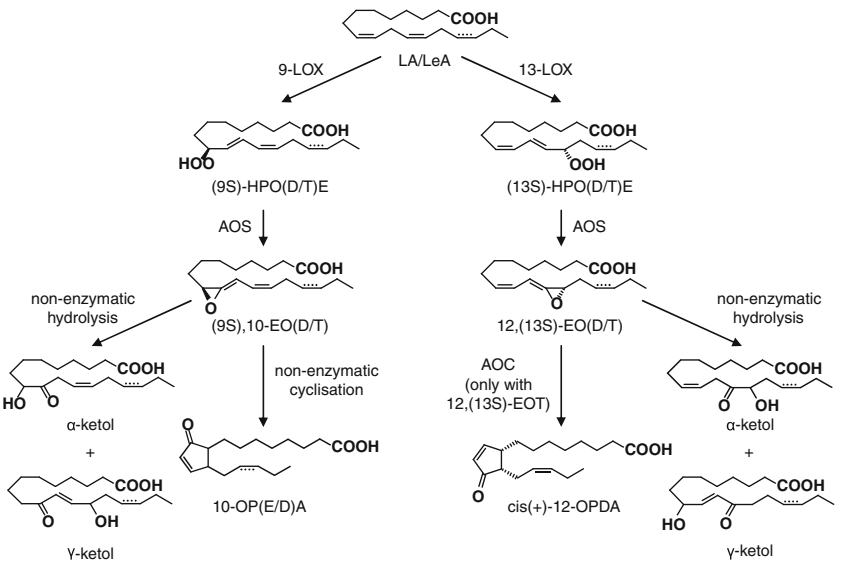


**Fig. 2** AOS-dependent metabolic route for linoleic (LA, without Δ15-double bond) and linolenic acid (LeA, with Δ15-double bond). AOC: Allene oxide cyclase, AOS: Allene oxide synthase, EO(D/T): Epoxy linole(n)ic acid, HPO(D/T)E: Hydroperoxy linole(n)ic acid, LOX: Lipoxygenase, OP(E/D)A: Oxo phyto(di)enoic acid

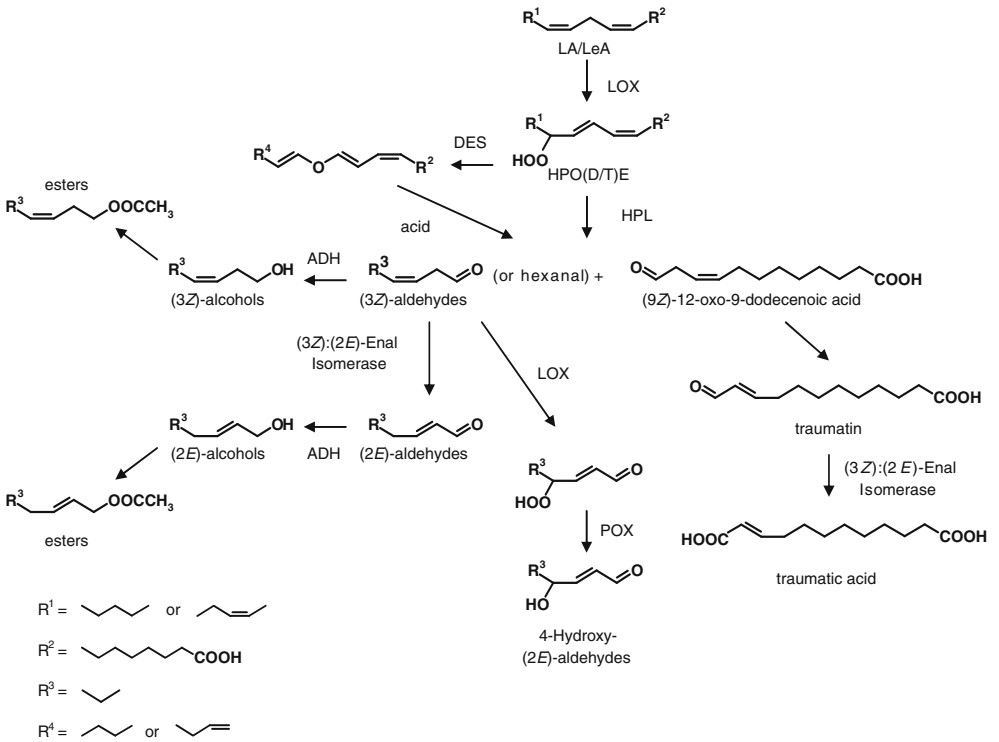


**Fig. 3** Metabolism of PUFAs leading to LOX-derived volatiles in plants. DES: Divinyl ether synthase, HPL: Hydroperoxide lyase, HPO(D/T)E: Hydroperoxy linole(n)ic acid, LA: Linoleic acid, LeA: Linolenic acid, LOX: Lipoxygenase, POX: Peroxygenase, ADH: Alcohol dehydrogenase

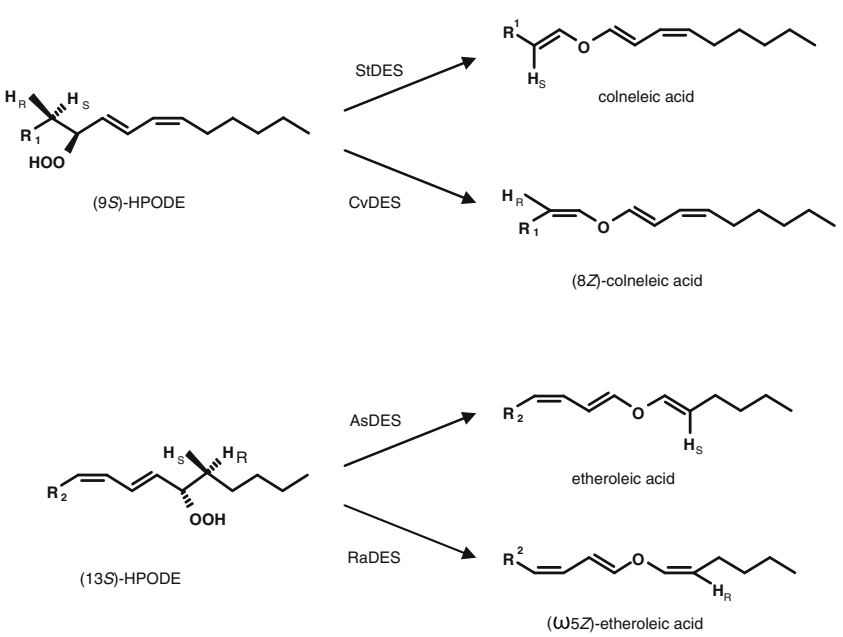


**Fig. 4** Stereospecificities of four divinyl ether synthases. AsDES: *Allium sativum*, CvDES: *Clematis vitalba*, HPODE: Hydroperoxy linoleic acid, RaDES: *Ranunculus acris*, StDES: *Solanum tuberosum*

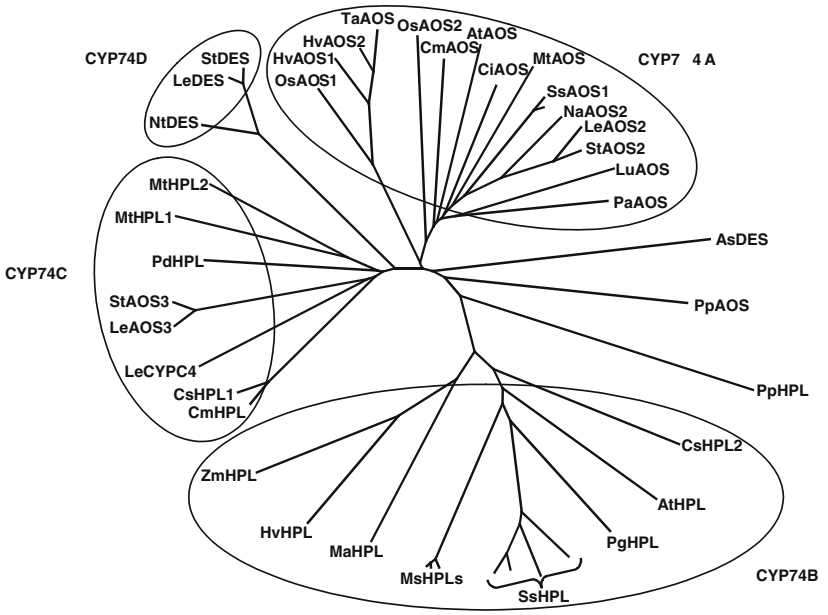


**Fig. 5** Phylogenetic tree analysis of the CYP74 enzyme family. Amino acid sequences were aligned using ClustalX. The phylogram was constructed using Treeview. Amino acid sequences corresponding to the following Acc.-No. were used for the analysis: *Arabidopsis thaliana*, AtAOS: CAA63266, AtHPL: AAC69871; *Allium sativum*, AsDES: CAI30435; *Citrus sinensis*, CiAOS: AAO72741; *Cucumis melo*, CmAOS: AAM66138, CmHPL: AAK54282; *Cucumis sativum*, CsHPL1: AAF64041, CsHPL2: AF229812; *Hordeum vulgare*, HvAOS1: CAB86384, HvAOS2: CAB86383, HvHPL: CAC82980; *Lycopersicom esculentum*, LeAOS2: AAF67141, LeAOS3: AAN76867, LeDES: AAG42261; *Linum usitatissimum*, LuAOS: AAA03353; *Musa acuminata*, MaHPL: CAB39331; *Medicago sativum*, MsHPLs: CAB54847, CAB54848, CAB54849; *Medicago truncatula*, MtAOS: CAC86897, MtHPL1: CAC86898, MtHPL2: CAC86899; *Nicotiana attenuata*, NaAOS2: CAC82911; *Nicotiana tabacum*, NtDES: AAL40900; *Oryza sativum*, OsAOS1: AAL38184, OsAOS2: AAP50956; *Parthenium argentatum*, PaAOS: CAA55025; *Prunus dulcis*, PdHPL: CAE18065; *Psidium guajava*, PgHPL: AAK15070; *Physcomitrella patens*, PpAOS: CAC86919, PpHPL: CAC86920; (Different) *Solanaceae* species, SsAOS1: CAB29735, CAB88032, SsHPL: AAF67142, AAA97465, CAC44040, CAC91565; *Solanum tuberosum*, StAOS2: CAB29736, StAOS3: CAI30876, StDES: CAC28152; *Triticum aestivum*, TaAOS: AAO43440; *Zea mais*, ZmHPL: from patent WO00/22145

these subfamilies (Fig. 5), it has been shown for PpHPL that it falls into the class of 9/13-HPLs (Stumpe et al. 2006a). In case of AOS it is very likely that the enzyme has a broad substrate specificity as well due to the high diversity of PUFAs found in this moss (Dembitsky 1993).

So far substrate specificity of most DES was only analysed using protein extracts of different plant organs. In potato and tomato a 9-hydroperoxide specific DES activity was described (Galliard and Phillips 1972). These results were supported by the characterization of the recombinant DES from these both plants (Itoh and Howe 2001; Stumpe et al. 2001). They prefer (9*S*,10*E*,12*Z*)-9-hydroperoxy-10,12-octadecadienoic acid (9-HPODE) whereas 13-hydroperoxides are only poor substrates. These enzymes were grouped into the CYP74D subfamily (Fig. 5). In leaves of *Ranunculus* plants and garlic bulbs, DES were found that use preferentially 13-hydroperoxides. In some algae, divinyl ether derived from $\omega$6-hydroperoxides of C18 and C20 fatty acids could be isolated (Gerwick 1994). So, one can assume that there are also DES with yet not described substrate specificities.

Analyses using artificial and different natural substrates indicate that the position of the hydroperoxide group in relation to the methyl end and not to the carboxyl end of the fatty acid is an important factor of the substrate specificity. Nevertheless the carboxy group plays also a role since methylation reduces often the activity, to some extend *N*-acyl(ethanol)amines are better substrates (Kandzia et al. 2003; Shrestha et al.

2002). Taken together it seems that free fatty acid derivatives and *N*-acyl(ethanol)amines are the preferred substrates of CYP74-enzymes (van der Stelt et al. 2000), but LOX-derived fatty acid hydroperoxides esterified to glycerolipids have been discussed as substrates as well (Stelmach et al. 2001). An influence of the pH is also discussed in respect to the amount of carboxylate anion in relation to non-dissociated carboxyl group.

## Further metabolism of CYP74 products

HPLs are key enzymes in the production of PUFA-derived volatiles (Gill and Valivety 1997a, b) (Fig. 3). Hydroperoxy PUFAs may be cleaved by HPL directly leading to short chain (3*Z*)-aldehydes which are transformed to (2*E*)-aldehydes via an enal isomerase or by mild acid catalysed isomerization (Noordermeer et al. 2001). Another way for the formation of such compounds is an acidic hydrolysis of divinyl ethers (Caldelari and Farmer 1998; Galliard et al. 1974). All aldehydes can undergo different reaction steps like reduction to alcohols (Noordermeer et al. 2001). They may react further with small organic acids such as acetic acid to the corresponding esters. Alternatively, the (3*Z*)-aldehydes may also be oxidized to 4-hydroxy-(2*E*)-aldehydes via LOX and peroxygenase reactions (Gardner and Hamberg 1993; Noordermeer et al. 2000; Schneider et al. 2001). In addition short chain alcohols may be directly formed by certain LOX forms. Recently, the reaction has been described for a linoleate 13-LOX from *Physcomitrella patens* (Senger et al. 2005). The other cleavage product of both reactions, the $\omega$-oxo fatty acid, can undergo isomerization and oxidation steps to dicarboxilic acid. By this way, (9*Z*)-12-oxo-9-dodecenoic acid derived from 13-hydroperoxides of C18 PUFAs is further converted into (10*E*)-12-oxo-10-dodecenoic acid (traumatin) and (10*E*)-dodecendioic acid (traumatic acid; Fig. 3). Their names derive from the observation that both substances may be involved in wound healing processes of plants (Zimmerman and Coudron 1979).

The unstable AOS reaction products, allene oxides, may be non-enzymatically hydrolysed into $\alpha$-ketol, $\gamma$-ketol and cyclopentenone fatty acids (Fig. 2). This chemical cyclization process is not restricted to allene oxides harbouring a double bond in $\beta,\gamma$-position to the epoxy group as it was assumed in former times (Grechkin 1994). Recent analyses of reaction products of recombinant AOS from tomato and potato as well as protein extracts of potato show an additional cyclopentenone fatty acid as a minor compound in each case that is derived from the allene oxide of 9-HPODE (Hamberg 2000; Itoh et al. 2002; Stumpe et al. 2006b). In addition to these non-specific chemical cyclization reactions an additional enzymatic cyclization reaction catalysed by AOC has been described (Hamberg 1988; Zimmerman and Feng 1977). It leads to a chiral cyclopentenone derivative (Ziegler et al. 1997). In vivo the cyclization of only two allene oxides that derive from 13-HPOTE and (7*Z*,9*E*,11*S*,13*Z*)-11-hydroperoxy-7,9,13-hexadecatrienoic acid, respectively, have been described. Their conversion leads to formation of OPDA and dinor-OPDA, respectively (Weber et al. 1997). Both cyclopentenoic acids are precursor of the plant hormone JA (Wasternack and Hause 2002).

## The reaction mechanism of enzymes from the CYP74 family

Information about the reaction mechanism of CYP74 enzymes is scarce till know. A common intermediate of the reactions catalysed by all CYP74 seem to be an epoxyallylic radical and/or carbocation formed from the acyl hydroperoxide (Feussner and Wasternack 2002; Grechkin 1998) (Fig. 6). The reactions catalysed by the different enzymes metabolizing this reaction intermediate however are different. HPL catalyse the cleavage of the carbon backbone after the attack of a hydroxyl group resulting in a hemiacetal (Grechkin and Hamberg 2004). Recently it has been shown that this hemiacetal is in fact the true product of the HPL reaction and not only an enzyme bound intermediate. Because of the high chemical instability the hemiacetal it is

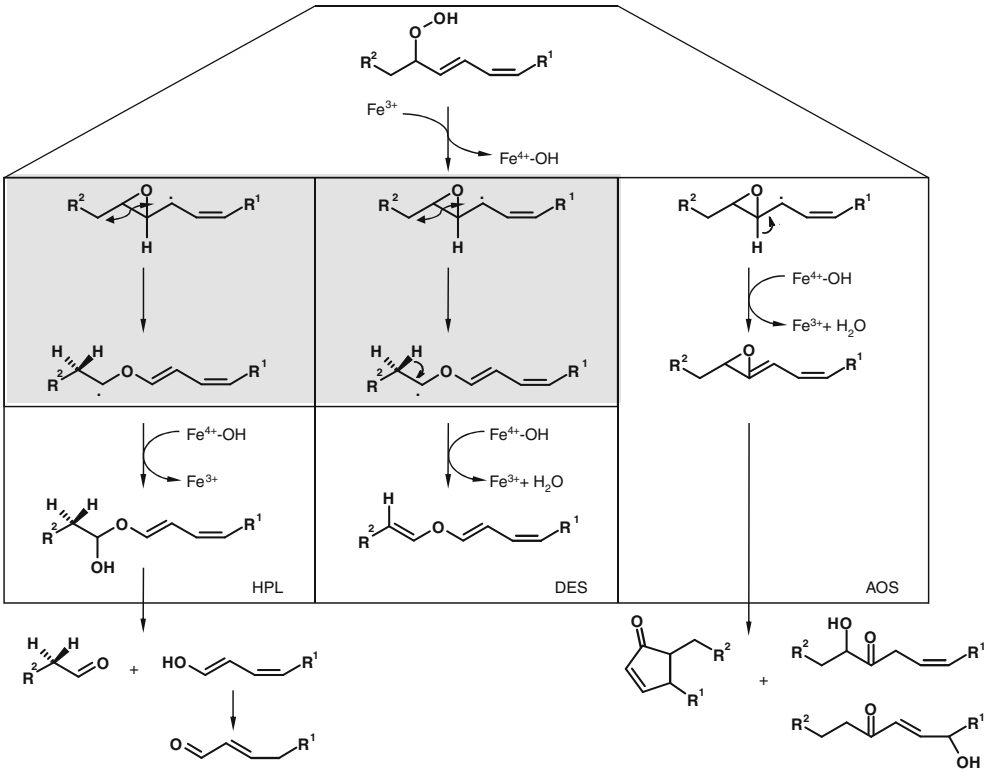


**Fig. 6** Proposed reaction mechanism of CYP74 enzymes according to (Feussner and Wasternack 2002; Grechkin 1998). Common feature is the epoxy allyl radical, which is stabilized in different ways. Reactions after homolytic cleavage of the hydroperoxides also possible as cations

decomposed to an aldehyde and an enol. The latter one is further converted to a second aldehyde function (Fig. 6). This mechanism is also supported by labelling experiments using [$^{18}O_2$]-13-HPOTE and [$^{18}O$]-water: The ether oxygen, as well as the hydroxyl oxygen of the hemiacetal product, is derived from the hydroperoxide group of the substrate, whereas no incorporation of the water oxygen was observed. These data also provided evidence for an exclusive homolytic reaction mechanism as it is also proposed for AOS (Song et al. 1993a). Being in agreement with the high catalytic activity of the CYP74 enzymes and the experimental evidence, it seems likely that all CYP74 enzymes act by a homolytic cleavage of the hydroperoxy fatty acid substrate and there is no formation of a carbocation during the reaction cycle.

DES catalyses also a cleavage of the carbon backbone. Here the intermediate is stabilized by deprotonation leading to the formation of divinyl ether. This step is stereospecific (Hamberg 2005). While DES of potato and garlic eliminate the pro-*R*-hydrogen resulting in a *trans* double bond, DES of *Clematis vitalba* and *Ranunculus acis* eliminate the pro-*S*-hydrogen resulting in a *cis* double bond in the product divinyl ether (Fig. 4).

Such a deprotonation occurs also during the AOS reaction to stabilize the initial epoxy allylic radical/cation. Here differences in the stereospecific deprotonation reaction by AOS are assumed as well which might explain differences in half life times of formed allene oxides and ratios of the *R*/*S* enantiomers of α-ketols after hydrolysis of allene oxides (Tijet and Brash 2002).

## Occurrence and intracellular localization of CYP74s

Whereas HPL and AOS are widespread in plant species (mono- and dicotyledons as well as

bryophytes like *Physcomitrella patens*), DES seem to be restricted to some algae and the genera *Solanum*, *Ranunculus*, as well as *Allium* (Fig. 5).

Numerous reports describe the activity of CYP74 enzymes in green tissues, often in chloroplasts. This localization seems to be likely because many cDNAs coding for CYP74 enzymes harbour at least a putative plastidic transit peptide. This is supported by immunocytochemical analysis e.g. in barley, potato and tomato leaves (Hause et al. 2003; Maucher et al. 2000; Stumpe et al. 2006b), by transient expression analysis of 9/13-HPL from *P. patens* in moss protoplasts (Stumpe et al. 2006a) and by in vitro import assays for 13-AOS and 13-HPL from tomato (Froehlich et al. 2001) as well as for three HPLs from rice (Chehab et al. 2006). For tomato it was shown that the 13-AOS is bound to the inner envelope and the 13-HPL to the outer envelope of plastids.

Analysing the transcription of AOS in *Arabidopsis thaliana* using promoter-GUS constructs, the GUS activity was observed in veins of young leaves and in matured pollen grains. In later stages the whole leaf was stained (Kubigsteltig et al. 1999). In tomato AOS protein was mainly detected in sieve elements (Hause et al. 2003). Nevertheless, CYP74 activity is also found in non-green tissues like fruits, seeds, seedlings, tubers and roots (Gardner 1991). Analysing the subcellular localization of StAOS3, which occurs mainly in non-green tissues, revealed targeting to the outer side of the envelope of plastids like amyloplasts or proplastids in such organs (Stumpe et al. 2006b). Another AOS was found to be the major protein of rubber particle in guayule (Pan et al. 1995) and recently there was a 9-HPL from almond characterized, which was targeted to lipid bodies (Mita et al. 2005). In general the picture emerges that the 13-LOX- and 9-LOX pathway may be separated within in the cell by the envelope membranes of the plastid: CYP74 enzymes that metabolize 13-LOX-derived fatty acid hydroperoxides seem to be localized at inner plastidial membranes whereby CYP74 enzymes that metabolize 9-LOX-derived fatty acid hydroperoxides seem to be localized either at the outer surface of the plastid (Feussner and Wasternack 2002; Howe and Schilmiller 2002) or even at extraplastidial membranes like it has been described for almond seeds or cucumber seedlings (Mita et al. 2005; Weichert et al. 2002). However, this model does neither explain the function nor the localization of the AOS from guayule. Therefore, further analysis of the intracellular distribution of other LOX pathway enzymes in this tissue is needed as well as a more detailed analysis of the biochemical properties of this AOS is necessary to explain its function in rubber particles.

## Physiological significance of CYP74 products

OPDA and JA are the major metabolites of the AOS pathway. They act as phytohormones that are involved in plant responses to stress and developmental processes. *Arabidopsis* mutants lacking both compounds have a reduced resistance to pathogens and herbivores. These mutants are also male sterile. These phenotypes can be rescued by the application of methyl jasmonate. For a more detailed discussion on the function of OPDA and jasmonates the reader is referred to other more specific reviews on this topic appearing recently (Berger 2002; Blée 2002; Rosahl and Feussner 2005; Schilmiller and Howe 2005; Wasternack and Hause 2002). Knowledge about the function of ketol fatty acids, the non-enzymatic products of the AOS pathway, is scarce. The analysis of extracts of *Lemna pauciostata* has yielded some hints for the physiological relevance of these compounds. Stress induces the production of the α-ketol derived from (9*S*,10*E*,12*Z*,15*Z*)-9-hydroperoxy-10,12,15-octade catrienoic acid (9-HPOTE) which reacts with norepinephrine to produce a flowering inducing compound (Yamaguchi et al. 2001). The α-ketol has also been linked to flower induction in *Pharbitis nil* (Suzuki et al. 2003).

The products of the HPL pathway, namely aldehydes, alcohols and esters, are compounds of the so-called ''green odor'' of plants. For example, the aroma of (3*Z*)-hexenal is known from fresh cut grass or tea and (3*Z*)-nonenal is typical for the aroma of cucumber fruits (Hatanaka 1996). In vitro aldehydes show also an antimicrobial effect so that the physiological function is

often addressed to plant defence against microbes (Noordermeer et al. 2001). Also discussed is that these compounds are a part of the volatile mixture that attract predator upon herbivore attack (Arimura et al. 2005). In transgenic potato plants lacking an HPL transcript, an increase in aphid performance was observed (Vancanneyt et al. 2001). In addition aldehydes seem to act also as signal compounds to induce transcripts of several defence related genes (Bate and Rothstein 1998; Kishimoto et al. 2006a, b) and it will be interesting to see whether the comparison of the *Arabidopsis* ecotypes Columbia and Landsberg will result in further details since Columbia is a natural HPL mutant (Duan et al. 2005). Moreover the HPL reaction seems to be an important metabolic pathway in algal defence reactions (Pohnert 2005).

Divinyl ethers may be inhibitors of LOXs and may play a role as phytoalexins since they reduce cytospore germination and mycelia growth of *Phytophthora infestans* in vitro (Grechkin 1995). These compounds seemed to have also a function in defence in plants. The amounts of divinyl ether as well as the transcript of DES increase in potato upon infection with *P. infestans* and *Pseudomonas syringae* (Stumpe et al. 2001; Weber et al. 1999). Plants with increased tolerance against the infection show a stronger accumulation of divinyl ether. Further analyses of transgenic plants are necessary to understand the function of these compounds. A recent study describes the effect of oxylipins for the first time on a large number of different plant pathogens by testing a large number of different substances (Prost et al. 2005).

PUFA oxidation is implicated in plant development and in responding to abiotic and biotic stresses. The past decade has seen a remarkable increase in our understanding in the involvement of the LOX pathway reactions and their physiological significance. The number and structural as well as functional diversity of LOX pathway enzymes enables the plant to appropriately respond to environmental challenges and not only to metabolize its lipids, but to use them as versatile signal molecules by introducing numerous new functionalities in these important biomolecules. The AOS-derived jasmonates and their octadecanoid precursors were the first oxylipins identified that have an assigned messenger function. In addition this knowledge is not only further increased by accumulating data on the molecular diversity of CYP74 enzymes as major catalysts of the LOX pathway, but the generation of CYP74 mutants, as well as ongoing overexpression and inactivation of genes encoding CYP74 enzymes or efforts to crystallize the purified enzymes, is expected to broaden our understanding of the biochemistry as well as on the physiological role of CYP74-derived metabolites in plant development and stress adaptation.

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## References

Arimura G-I, Kost C, Boland W (2005) Herbivore-induced, indirect plant defences. Biochim Biophys Acta 1734:91–111

Bate NJ, Rothstein SJ (1998) C-6-volatiles derived from the lipoxygenase pathway induce a subset of defense-related genes. Plant J 16:561–569

Berger S (2002) Jasmonate-related mutants of *Arabidopsis* as tools for studying stress signaling. Planta 214:497–504

Blée E (1998) Phytooxylipins and plant defense reactions. Prog Lipid Res 37:33–72

Blée E (2002) Impact of phyto-oxylipins in plant defense. Trends Plant Sci 7:315–322

Brash AR (1999) Lipoxygenases: occurrence, functions, catalysis, and acquisition of substrate. J Biol Chem 274:23679–23682

Caldelari D, Farmer EE (1998) A rapid assay for the coupled cell free generation of oxylipins. Phytochemistry 47:599–604

Chehab EW, Raman G, Walley JW, Perea JV, Banu G, Theg S, Dehesh K (2006) Rice hydroperoxide lyases with unique expression patterns generate distinct aldehyde signatures in *Arabidopsis*. Plant Physiol 141:121–134

Dembitsky VM (1993) Lipids of bryophytes. Prog Lipid Res 32:281–356

Duan H, Huang M-Y, Palacio K, Schuler MA (2005) Variations in *CYP74B2* (hydroperoxide lyase) gene expression differentially affect hexenal signaling in the Columbia and Landsberg *erecta* ecotypes of *Arabidopsis*. Plant Physiol 139:1529–1544

Feussner I, Wasternack C (2002) The lipoxygenase pathway. Annu Rev Plant Biol 53:275–297

Froehlich JE, Itoh A, Howe GA (2001) Tomato allene oxide synthase and fatty acid hydroperoxide lyase, two cytochrome P450s involved in oxylipin metabolism, are targeted to different membranes of chloroplast envelope. Plant Physiol 125:306–317

Galliard T, Phillips DR (1972) The enzymic conversion of linoleic acid into 9-(nona-1′,3′-dienoxy)non-8-enoic acid, a novel unsaturated ether derivate isolated from homogenates of *Solanum tuberosum* tubers. Biochem J 129:743–753

Galliard T, Wardale DA, Mathew JA (1974) The enzymic and non-enzymic degradation of colneleic acid, an unsaturated fatty acid ether intermediate in the lipoxygenase pathway of linoleic acid oxidation in potato (*Solanum tuberosum*) tubers. Biochem J 138:23–31

Gardner HW (1991) Recent investigations into the lipoxygenase pathway of plants. Biochim Biophys Acta 1084:221–239

Gardner HW (1996) Lipoxygenase as a versatile biocatalyst. J Am Oil Chem Soc 73:1347–1357

Gardner HW, Hamberg M (1993) Oxygenation of (3Z)-nonenal to (2E)-4-hydroxy-2-nonenal in the broad bean (Vicia-Faba L). J Biol Chem 268:6971–6977

Gerwick WH (1994) Structure and biosynthesis of marine algal oxylipins. Biochim Biophys Acta 1211:243–255

Gill I, Valivety R (1997a) Polyunsaturated fatty acids, part 1: occurrence, biological activities and applications. Trends Biotechnol 15:401–409

Gill I, Valivety R (1997b) Polyunsaturated fatty acids, part 2: biotransformations and biotechnological applications. Trends Biotechnol 15:470–478

Grechkin A (1995) Physiologically active products of plant lipoxygenase pathway. In: Kader J-C, Mazliak P (eds) Plant lipid metabolism. Kluwer Academic Publishers, Dordrecht, pp 274–279

Grechkin A (1998) Recent developments in biochemistry of the plant lipoxygenase pathway. Prog Lipid Res 37:317–352

Grechkin AN (1994) Cyclization of natural allene oxide fatty acids. The anchimeric assistance of $\beta,\gamma$-double bond beside the oxirane and the reaction mechanism. Biochim Biophys Acta 1213:199–206

Grechkin AN, Hamberg M (2004) The ''heterolytic hydroperoxide lyase'' is an isomerase producing a short-lived fatty acid hemiacetal. Biochim Biophys Acta 1636:47–58

Hamberg M (1988) Biosynthesis of 12-oxo-10,15(Z)-phytodienoic acid: identification of an allene oxide cyclase. Biochem Biophys Res Comm 156:543–550

Hamberg M (2000) New cyclopentenone fatty acids formed from linoleic and linolenic acids in potato. Lipids 35:353–363

Hamberg M (2005) Hidden stereospecificity in the biosynthesis of divinyl ether fatty acids. FEBS J 272:736–743

Hatanaka A (1996) The fresh green odor emitted by plants. Food Rev Int 12:303–350

Hause B, Hause G, Kutter C, Miersch O, Wasternack C (2003) Enzymes of jasmonate biosynthesis occur in tomato sieve elements. Plant Cell Physiol 44:643–648

Howe GA, Schilmiller AL (2002) Oxylipin metabolism in response to stress. Curr Opin Plant Biol 5:230–236

Itoh A, Howe GA (2001) Molecular cloning of a divinyl ether synthase: identification as a CYP74 cytochrome P450. J Biol Chem 276:3620–3627

Itoh A, Schilmiller AL, McCaig BC, Howe GA (2002) Identification of a jasmonate-regulated allene oxide synthase that metabolizes 9-hydroperoxides of linoleic and linolenic acids. J Biol Chem 277:46051–46058

Kandzia R, Stumpe M, Berndt E, Szalata M, Matsui K, Feussner I (2003) On the specificity of lipid hydroperoxide fragmentation by fatty acid hydroperoxide lyase from *Arabidopsis thaliana*. J Plant Physiol 160:803–809

Kishimoto K, Matsui K, Ozawa R, Takabayashi J (2006a) Components of C6-aldehyde-induced resistance in *Arabidopsis thaliana* against a necrotrophic fungal pathogen, *Botrytis cinerea*. Plant Sci 170:715–723

Kishimoto K, Matsui K, Ozawa R, Takabayashi J (2006b) ETR1-, JAR1- and PAD2-dependent signaling pathways are involved in C6-aldehyde-induced defense responses of *Arabidopsis*. Plant Sci 171:415–423

Kubigsteltig I, Laudert D, Weiler EW (1999) Structure and regulation of the *Arabidopsis thaliana* allene oxide synthase gene. Planta 208:463–471

Matsui K (1998) Properties and structures of fatty acid hydroperoxide lyase. Belgian J Bot 131:50–62

Matsui K, Miyahara C, Wilkinson J, Hiatt B, Knauf V, Kajiwara T (2000a) Fatty acid hydroperoxide lyase in tomato fruits: cloning and properties of a recombinant enzyme expressed in *Escherichia coli*. Biosci Biotechnol Biochem 64:1189–1196

Matsui K, Ujita C, Fujimoto S, Wilkinson J, Hiatt B, Knauf V, Kajiwara T, Feussner I (2000b) Fatty acid 9- and 13-hydroperoxide lyases from cucumber. FEBS Lett 481:183–188

Maucher H, Hause B, Feussner I, Ziegler J, Wasternack C (2000) Allene oxide synthases of barley (*Hordeum vulgare* cv. Salome): tissue specific regulation in seedling development. Plant J 21:199–213

Mita G, Quarta A, Fasano P, De Paolis A, Di Sansebastiano GP, Perrotta C, Iannacone R, Belfield E, Hughes R, Tsesmetzis N, Casey R, Santino A (2005) Molecular cloning and characterization of an almond 9-hydroperoxide lyase, a new CYP74 targeted to lipid bodies. J Exp Bot 56:2321–2333

Noordermeer MA, Feussner I, Kolbe A, Veldink GA, Vliegenthart JF (2000) Oxygenation of (3*Z*)-alkenals to 4-hydroxy-(2*E*)-alkenals in plant extracts: a non-enzymatic process. Biochem Biophys Res Commun 277:112–116

Noordermeer MA, Veldink GA, Vliegenthart JF (2001) Fatty acid hydroperoxide lyase: a plant cytochrome P450 enzyme involved in wound healing and pest resistance. ChemBioChem 2:494–504

Pan Z, Durst F, Werck-Reichhart D, Gardner HW, Camara B, Cornish K, Backhaus RA (1995) The major protein of guayule rubber particles is a cytochrome P450. J Biol Chem 270:8487–8494

Pohnert G (2005) Diatom/copepod interactions in plankton: the indirect chemical defense of unicellular algae. ChemBioChem 6:1–14

Prost I, Dhondt S, Rothe G, Vicente J, Rodriguez MJ, Kift N, Carbonne F, Griffiths G, Esquerre-Tugaye M-T, Rosahl S, Castresana C, Hamberg M, Fournier J (2005) Evaluation of the antimicrobial activities of plant oxylipins supports their involvement in defense against pathogens. Plant Physiol 139:1902–1913

Rosahl S, Feussner I (2005) Oxylipins. In: Murphy D (ed) Plant lipids. Blackwell Publishing Ltd., Oxford, UK, pp 329–354

Schilmiller AL, Howe GA (2005) Systemic signaling in the wound response. Curr Opin Plant Biol 8:369–377

Schneider C, Tallman KA, Porter NA, Brash AR (2001) Two distinct pathways of formation of 4-hydroxynonenal: mechanisms of non-enzymatic transformation of the 9- and 13-hydroperoxides of linoleic acid to 4-hydroxyalkenals. J Biol Chem 276:20831–20838

Senger T, Wichard T, Kunze S, Göbel C, Lerchl J, Pohnert G, Feussner I (2005) A multifunctional lipoxygenase with fatty acid hydroperoxide cleaving activity from the moss *Physcomitrella patens*. J Biol Chem 280:7588–7596

Shrestha R, Noordermeer MA, Van der Stelt M, Veldink GA, Chapman KD (2002) *N*-Acylethanolamines are metabolized by lipoxygenase and amidohydrolase in competing pathways during cottonseed imbibition. Plant Physiol 130:391–401

Song WC, Baertschi SW, Boeglin WE, Harris TM, Brash AR (1993a) Formation of epoxyalcohols by a purified allene oxide synthase. Implications for the mechanism of allene oxide synthesis. J Biol Chem 268:6293–6298

Song WC, Funk CD, Brash AR (1993b) Molecular cloning of an allene oxide synthase – a cytochrome-P450 specialized for the metabolism of fatty acid hydroperoxides. Proc Natl Acad Sci USA 90:8519–8523

Stelmach BA, Müller A, Hennig P, Gebhardt S, Schubert-Zsilavecz M, Weiler EW (2001) A novel class of oxylipins, *sn*1-*O*-(12-Oxophytodienoyl)-*sn*2-*O*-(hexadecatrienoyl)-monogalactosyl diglyceride, from *Arabidopsis thaliana*. J Biol Chem 276:12832–12838

Stumpe M, Bode J, Göbel C, Wichard T, Schaaf A, Frank W, Frank M, Reski R, Pohnert G, Feussner I (2006a) Biosynthesis of C9-aldehydes in the moss *Physcomitrella patens*. Biochim Biophys Acta 1761:301–312

Stumpe M, Göbel C, Demchenko K, Hoffmann M, Klosgen RB, Pawlowski K, Feussner I (2006b) Identification of an allene oxide synthase (CYP74C) that leads to formation of α-ketols from 9-hydroperoxides of linoleic and linolenic acid in below-ground organs of potato. Plant J 47:883–896

Stumpe M, Kandzia R, Göbel C, Rosahl S, Feussner I (2001) A pathogen-inducible divinyl ether synthase (*CYP74D*) from elicitor-treated potato suspension cells. FEBS Lett 507:371–376

Suzuki M, Yamaguchi S, Iida T, Hashimoto I, Teranishi H, Mizoguchi M, Yano F, Todoroki Y, Watanabe N, Yokoyama M (2003) Endogenous α-ketol linolenic acid levels in short day-induced cotyledons are closely related to flower induction in *Pharbitis nil*. Plant Cell Physiol 44:35–43

Tijet N, Brash AR (2002) Allene oxide synthases and allene oxides. Prostaglandins Lipid Mediators 68–69:423–431

van der Stelt M, Noordermeer MA, Kiss T, Van Zadelhoff G, Merghart B, Veldink GA, Vliegenthart JF (2000) Formation of a new class of oxylipins from *N*-acyl(ethanol)amines by the lipoxygenase pathway. Eur J Biochem 267:2000–2007

Vancanneyt G, Sanz C, Farmaki T, Paneque M, Ortego F, Castanera P, Sanchez-Serrano JJ (2001) Hydroperoxide lyase depletion in transgenic potato plants leads to an increase in aphid performance. Proc Natl Acad Sci USA 98:8139–8144

Wasternack C, Hause B (2002) Jasmonates and octadecanoids: signals in plant stress responses and development. Prog Nucleic Acid Res Mol Biol 72:165–221

Weber H, Chetelat A, Caldelari D, Farmer EE (1999) Divinyl ether fatty acid synthesis in late blight-diseased potato leaves. Plant Cell 11:485–493

Weber H, Vick BA, Farmer EE (1997) Dinor-oxo-phytodienoic acid: a new hexadecanoid signal in the jasmonate family. Proc Natl Acad Sci USA 94:10473–10478

Weichert H, Kolbe A, Kraus A, Wasternack C, Feussner I (2002) Metabolic profiling of oxylipins in germinating cucumber seedlings – lipoxygenase-dependent degradation of triacylglycerols and biosynthesis of volatile aldehydes. Planta 215:612–619

Yamaguchi S, Yokoyama M, Iida T, Okai M, Tanaka O, Takimoto A (2001) Identification of a component that induces flowering of *Lemna* among the reaction products of α-ketol linolenic acid (FIF) and norepinephrine. Plant Cell Physiol 42:1201–1209

Ziegler J, Hamberg M, Miersch O, Parthier B (1997) Purification and characterization of allene oxide cyclase from dry corn seeds. Plant Physiol 114:565–573

Zimmerman DC, Coudron CA (1979) Identification of traumatin, a wound hormone, as 12-oxo-trans-10-dodecenoic acid. Plant Physiol 63:536–541

Zimmerman DC, Feng P (1977) Characterization of a prostaglandin-like metabolite of linolenic acid produced by a flaxseed extract. Lipids 13:313–316