OXYLIPINS AND RELEVANT ENZYMES IN PLANT DEFENCE

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RESUME

The oxylipins form a class of secondary metabolites generated from polyunsaturated fatty acids (PUFAs) *via* the so-called lipoxygenase (LOX) pathway. This review concentrates on their role in plant defense against microbial invasion, with a special emphasis on the outcomes of functional genetics approaches in the branches initiated by CYP74 enzymes downstream of LOX action. These include allene oxide synthases (AOSs), hydroperoxide lyases (HPLs) and divinyl ether synthases (DESs). It also covers cross-talk with other signal molecules implicated in the defense response.

SUMMARY

1.0. *General introduction*

As sessile organisms, plants are continuously exposed to external stresses impinging on their development and growth. These can be either abiotic or biotic in nature, and their variation in duration, intensity and frequency generates a wide range of cellular responses. In combination, different stress agents can induce a very different response from that generated by one stress acting alone. The response can be quite specific, in terms of place (individual organs or tissue), time (developmental stage) and genotype. Unable to escape interaction with all the potentially pathogenic (micro)organisms with which they come into contact, plants have evolved a set of constitutive and inducible defenses. Beyond pre-formed structural and chemical barriers (such as the cell wall, the cuticle, glucosides, tannins, phenols, resins, phytoanticipins etc), inducible responses play a key role in pathogen containment both in host and non-host interactions [*i.e.* both with adapted and non-adapted pathogens, as well as with non pathogens; (de Wit 1995)]. Anti-microbial metabolites and proteins (such as cell-wall degrading enzymes, certain peptides released from germinating seeds, lectins and inhibitors of various enzymes) are implicated in both compatible and incompatible interactions, although with variable timing, intensity and, of course, efficacy. The initiation of an inducible response requires the activation and repression of a suite of defense-related genes and gene products at the transcriptional and post-transcriptional level. This in turn depends on effective “non-self” perception and downstream signal transduction, in which processes such as the opening of ion channels and changes in the phosphorylation status of a number of key proteins are involved. In one extreme reaction of affected cells, referred to as the “hypersensitive response” (HR), a genetically defined cell-death program (a form of apoptosis) is set in motion, and this is particularly effective for the physical isolation of a phytopathogen (Greenberg and Yao 2004).

This chapter describes the involvement of a group of fatty acid-derived molecules, produced through the so called “oxylipin pathway”, in inducible plant defense against biotic stress. Since several excellent recent reviews have been published on this topic, the scope is limited here to an overview of the literature, and concentrates on recent advances in the understanding of the role of certain metabolites within the very diverse oxylipin family in plant defense against microbes.

2.0. *The oxylipin pathway*

Various oxygenated fatty acids and their derived metabolites, collectively termed oxylipins, are involved not only in metabolism, but also in the response to biotic and abiotic stress (Brash 1999). The oxidation of polyunsaturated fatty acids (PUFAs) is common to all eukaryotes studied to date, regardless of whether they be fungi, algae, plants or animals. The process occurs during lipid synthesis and recycling, but is also used for the production of bio-active compounds. The synthesis of PUFA hydroperoxides is an early and necessary step in the metabolism of all oxylipins (which comprise PUFA hydroperoxides and their derivatives), and is among the early events to be triggered by environmental stress (Brash 1999).

Oxidized PUFAs can be generated *via* chemical oxidation, often called auto-oxidation (Rusterucci et al., 1999; Spiteller et al., 2001), but the oxylipins relevant in plant defense are mostly enzymatically derived from the fatty acids released from either phospho- or glycolipids, either *via* the action of phospholipases (PLs) or acylester hydrolases. Lipoxygenases (LOXs), and to a lesser extent dioxygenases (α-DOXs) too, are involved in this process. α-DOXs metabolize linoleic acid (LA, $18{:}2^{\Delta 9Z,12Z}$, where x:yΔz is a fatty acid chain of x carbons, containing y double bonds in position(s) z counting from the carboxyl end) and α-linolenic acid (LnA, $18{:}3^{\Delta 9Z,12Z,15Z}$) to produce, respectively, the reactive 2-hydroperoxy octadeca(di/tri)enoic acids [2-HPO(D/T)]; these are either converted to their corresponding anti-microbial 2-hydroxy octadeca(di/tri)enoic acids [2-HO(D/T)], or they undergo a non-enzymatic decarboxylation to generate C18-1 odd-numbered aldehyde derivatives, and by oxidation to the corresponding free fatty acid (Hamberg et al., 1999; Hamberg et al., 2003; Saffert et al., 2000).

More commonly, the conversion of LA and LnA into their corresponding C18 hydroperoxy fatty acids [HPO(D/T)s] is achieved by specific LOXs, specialized for the conversion of LA and LnA (Feussner et al., 1997; Rusterucci et al., 1999). Depending on the site of action on the C18 PUFA chain, plant LOXs are classified as 9- or 13-LOXs, which produce either 9- or 13-HPODs or HPOTs from, respectively, the di- or tri-unsaturated fatty acids (see §3.1 and Fig. 1). These hydroperoxides are reactive molecules, and serve as substrates for at least six other enzymes including the LOXs themselves (Blée 2002; Feussner and Wasternack 2002). As a result of the numerous catalytic mechanisms deployed, plant

oxylipins represent a wide and polymorphic family of secondary metabolites (Fig. 2), classified into 9- and 13-LOX-derived compounds.

The 9- and 13-HPO(D/T)s can be metabolized (Fig. 3) by:

- peroxygenase (POX), followed by hydration by an epoxide hydrolase (Blée 1998) to generate either epoxy-, epoxy alcohol-, trihydroxy- or hydroxy- fatty acids (Blée 2002; Weichert et al., 1999)
- epoxy alcohol synthase (EAS) (Hamberg et al., 1999), followed by hydration by an epoxide hydrolase (Blée 1998) to generate epoxy hydroxy- or trihydroxy- fatty acids
- LOX, which catalyzes their dehydration to keto(di/tri)enes of fatty acids [KO(D/T)s] under certain environmental conditions such as low oxygen pressure (Vollenweider et al., 2000)
- hydroperoxide lyase (HPL), which generates unstable hemiacetals. These dissociate rapidly into short-chain aldehydes (C_6– or C_9–) and the corresponding C_{12}– and C_9–ω-oxo fatty acids (Grechkin 2002; Grechkin and Hamberg 2004)
- divinyl ether synthase (DES), forming divinyl ethers (DVEs) (Grechkin 2002)
- allene oxide synthase (AOS) (Tijet and Brash 2002), which catalyzes their dehydration into unstable allene oxides. These can undergo non-enzymatic hydrolysis to form α– and γ–ketols and racemic cyclopentenols, or in the case of 13-HPOT, be cyclized by allene oxide cyclase (AOC) and further modified to generate prostaglandin-like molecules such as 12-oxophytodienoic acid (oPDA). 12-oPDA itself can be reduced and β–oxidized into the plant hormone jasmonic acid (JA) (Vick and Zimmerman 1983)

In addition to the oxylipins derived from C18-PUFAs, a similar set of metabolites derived from roughanic acid ($16{:}3^{\Delta 7Z,10Z,13Z}$) has been described (Andersson et al., 2006; Kourtchenko et al., 2007). However, these will be not discussed here, in order to avoid over-complicating the picture. Quantitative, spatial and temporal variation, as well as qualitative differences among oxylipins produced under various conditions by different plants, are known as an “oxylipin signature” (Weber et al., 1999; Weber et al., 1997). An excellent review covering the metabolic routes to the structural diversity of oxylipins has recently been published (Wasternack 2007).

3.0. *Oxylipins and plant defense; general features*

The role of oxylipins in pathogen defense has been elucidated by a number of experimental approaches, although the individual contributions of each oxylipin and the mechanisms *via* which they exert their activity have mostly remained obscure, with a few exceptions. Many oxylipins have wide anti-microbial activity, presumably thanks to their chemical and physical characteristics, rather than to any interaction with a specific molecular target (Prost et al., 2005). The 13-HPOT and 13-HOT (Graner et al., 2003), the 9-DVEs colneleic acid (CA) and colnelenic acid (CnA) (Weber et al., 1999), and several other epoxy- or poly-hydroxy fatty acids (Blée 1998) all show *in vitro* anti-bacterial, anti-fungal or anti-oomycete activity (Prost et al., 2005). Intriguingly, the toxicity level - and therefore presumably the mode of action - of each oxylipin depends on which microorganism is challenged (Prost et al., 2005). Their toxicity against fungi and oomycetes may reflect their interference with the pathogen's metabolism and/or signal transduction, as a result of their having a structural affinity with endogenous eicosanoids and oxylipins important for the growth, differentiation and life cycle of these microorganisms (Kock et al., 2003; Noverr et al., 2003; Tsitsigiannis et al., 2004). A cross-reaction between PUFA derivatives and membrane lipids cannot be excluded, because of their similar chemical properties and origin. The mechanism of action of *cis*-9-eptadecenoic acid has been linked to its capacity to penetrate the fungal lipid bilayer, causing membrane collapse and subsequent cell death (Avis and Belanger 2001, 2002). At the whole-plant level, experimental evidence for the contribution of particular oxylipins to plant defense is often correlative - for example by demonstrating that oxylipin abundance increases in stressed plants, or that exogenous application alters the plant's susceptibility to pathogen infection (Devoto and Turner 2003; Göbel et al., 2002).

The antimicrobial properties of many oxylipins gives no handle as to their role as signaling molecules *in planta.* Some, however, were shown to do so by modulating the expression of defense-related genes (La Camera et al., 2004). The following are thought to function as signal molecules:

- 9- and 13-HPO(D/T)s produced by LOXs (Knight et al., 2001; Montillet et al., 2005; Rusterucci et al., 1999)

- oxylipins generated by α-DOX (Hamberg et al., 2003; Ponce de Leon et al., 2002)
- several 13-LOX derivatives, *e.g.* 12-oPDA, JA and some JA-derivatives (MeJA, JA-Ile, ...) (Balbi and Devoto 2008)
- aldehydes produced by 13-HPLs (Bate and Rothstein 1998; Farmer et al., 2003; Kandzia et al., 2003; Kishimoto et al., 2005)
- 13-HOD and 13-HOT (Vollenweider et al., 2000; Weichert et al., 1999)

The reactivity of all these molecules, and their effects on metabolic processes are reminiscent of those of hydrogen peroxide (H_2O_2). Indeed, as already alluded to above, many oxylipins are themselves hydroperoxides. Hydrogen peroxide is a reactive oxygen species (ROS), formed as a (by-)product of cellular metabolism. It acts as a signal molecule at low concentrations, but is toxic at higher levels (Apel and Hirt 2004). The mechanism of action of some “bio-active” oxylipins (for example 12-oPDA and 13-ketotrienes) is related to their electrophilic property, which appears to be responsible for damaging plant cells and the consequent expression of defense-related genes (Almeras et al., 2003; Farmer et al., 2003).

Of the molecules listed above, the most extensively studied has been JA, along with its derivatives and precursors (collectively called jasmonates, JAs). The role of the JAs in defense is now well established, although it remains far from being completely elucidated. Signaling networks based on JA are particularly complex; most information on them is being collected in the model plant *Arabidopsis thaliana*, in which several mutants, compromised for various signaling steps both upstream of biosynthesis and downstream of perception, have been isolated. These have allowed the identification of some critical genetic components, and a view of the extent of the cross-talk which occurs with other defense signaling pathways, namely those based on ethylene (ET) and salicylic acid (SA). JA is also one of the key players in both ISR (Induced Systemic Resistance; (Heil and Bostock 2002)) and SAR (Systemic Acquired Resistance; (Loake and Grant 2007)). For further information regarding these topics, see §5.0 and the excellent review by Balbi and Devoto (Balbi and Devoto 2008). An impairment in the production of certain oxylipins achieved by genetic modification has also strengthened the case for their involvement in the resistance of plants to pathogen attack, as detailed below.

3.1. *Lipoxygenases (LOXs)*

LOXs are non-heme, iron-containing fatty acid oxygenases which catalyze the regio- and stereo-specific dioxygenation of PUFAs containing a (1*Z*,4*Z*)-pentadiene system - for example, LA and LnA in plants, and arachidonic acid (C20:4) in mammals (Feussner and Kuhn 2000). Recently, LOXs have also been identified in bacteria (Koeduka et al., 2007; Lang and Feussner 2007; Lang et al., 2008; Schneider et al., 2007a; Vance et al., 2004). The classification of plant LOXs is based on the stereo-specific oxidation of LA. Two independent events occur during catalysis: the first is a de-protonation at C11 (Feussner and Kuhn 2000); the second the selection of the site of oxygen insertion, which normally occurs at position [+2] or [-2] with respect to C11, leading to dioxygenation of C9 or C13 (Fig. 4) (Feussner and Wasternack 2002; Schneider et al., 2007b).

Initially, two models were elaborated to explain the site-specific catabolism of LOXs. The first, the “space-related hypothesis”, was developed in the context of mammalian LOXs. It proposed that the methyl group at the extremity of the aliphatic chain is the first to interact with the inner part of LOX catalytic site (Gillmor et al., 1997; Sloane et al., 1995). The position of the carbon to be oxygenated is then determined by the length of the pocket enveloping the fatty acid chain. This model, however, does not accommodate the primary reaction at the C11 level (Fig. 4) (Feussner and Wasternack 2002). The second “orientation-dependent” model was also based on the orientation of the substrate, but suggested a head-to-tail entrance to the binding pocket, in which the methyl group was always the first to enter the binding pocket of the 13-LOXs, while the carboxylic group leads in 9-LOXs (Gardner 1989). Selectivity is then based on the conformation of the entrance of the catalytic groove. In this model, C11 is the key carbon atom and oxygenation at [+2] or at [-2] depends on the isoenzyme (Fig. 4). Recent reports have sought to explain the reaction dynamics as a combination of both models (Hornung et al., 1999). For a current review on the catalytic specificities and physiological roles of plant LOXs, see Liavonchanka and Feussner (2006) (Liavonchanka and Feussner 2006).

A more comprehensive classification of LOXs has been proposed on the basis of their primary structure. Two major LOX subfamilies have been distinguished: type-1, consisting of highly similar sequences (>75%) all lacking a plastid transit peptide, and type-2, consisting of members of low similarity (about 35%), but possessing a transit

peptide (Wasternack and Hause 2002). The implications of the sub-cellular localization of oxylipin biosynthetic enzymes are discussed elsewhere in this chapter (§4.0).

Members of the LOX family are involved at various stages of vegetative and propagative plant development (Creelman and Mullet 1997; Liavonchanka and Feussner 2006), but some are also associated with the response to biotic and abiotic stress. The variety of physiological processes within which LOX activity is deployed is reflected in the size of the gene family, which typically comprises numerous isoforms, each of which is differentially regulated in terms of its expression and sub-cellular localization (Rosahl and Feussner 2005). The free PUFAs, substrates of type-1 LOXs, are derived mainly by the action of PLs on plasma-membrane phospholipids (Joyard et al., 1998); as a result, PLs become key regulators of the LOX pathways. Alternatively the substrate is generated from free stromal fatty acids which are released from glyco- or phospholipids by the action of non-specific acyl-lipid hydrolases such DAD1 or patatins (Dhondt et al., 2000; Ishiguro et al., 2001). Finally, cytosolic LOXs (type-1 LOXs) can also utilize esterified PUFAs or PUFA-CoA (Brash et al., 1987; Dhondt et al., 2000; Larson and Graham 2001; Maccarrone et al., 1994). PL activity increases rapidly after the onset of stress or treatment with elicitors (Narvaez-Vasquez et al., 1999; Roy et al., 1995), and in petunia, patatin activity has been shown to be necessary for the expression of full resistance to a fungal and a bacterial pathogen (Zahn et al., 2005).

Strong induction of LOX-encoding genes occurs in response to both wounding and pest attack, both in mono- and dicotyledonous plants. The stronger and earlier occurring transcription of specific LOX isoforms in incompatible as opposed to compatible interactions suggests a role for them in defense (Gao et al., 2008a; Gao et al., 2008b; Koch et al., 1992; Kolomiets et al., 2000; Peng et al., 1994; Véronési et al., 1996). In addition to such correlative evidence, several loss- and/or gain-of-function transgenic strategies have been deployed to causally link LOX activity with wild-type resistance to various pests. As an example, the depletion of a certain 9–LOX impaired the resistance of a *Nicotiana tabacum* L. line to an avirulent race of the oomycete *Phytophthora parasitica* var *nicotianae* (Rancé et al., 1998). Conversely, over-expression of this same 9–LOX conferred resistance to a virulent race of the same pathogen (Mène-Saffrané et al., 2003). The reduced expression in potato and *Nicotiana attenuata* of specific 13-LOX isoforms has

been associated with improved performance of herbivores (Halitschke and Baldwin 2003; Royo et al., 1999).

Finally, LOXs contribute to basal susceptibility in certain pathosystems. For example, the maize gene *ZmLOX3* encodes a 9-LOX, the metabolic products of which suppress JA, ET and SA-dependent pathways; thus, *lox3* mutants display a higher level of resistance to *Colletotrichum graminicola*, *Cochliobolus heterostrophus*, *Fusarium verticillioides* (and correspondingly, less fumonisin contamination of the kernels). Sporulation in these pathogens is partly compromised in mutant tissues, suggesting that plant oxylipin metabolism is required for fungal pathogenesis, including disease development and the production of mycotoxin (Gao et al., 2008a; Gao et al., 2007). The same LOX isoform, however, is needed for wild-type resistance to root-knot nematodes, possibly *via* the phenylalanine-ammonia lyase (PAL) metabolic pathway (Gao et al., 2008a).

Altogether, evidence is accumulating that LOXs are major actors in plant defense, thanks to the signaling and anti-microbial functions of their products, and to the fact that they are typically induced by the presence of stress. The altered fitness of plants compromised in the expression of various LOX isoforms lends genetic support to the hypothesis. However, because of the large number of metabolic routes initiated by LOXs, these phenotypes are seldom informative regarding the relative contribution of individual branches of the oxylipin pathway. This limitation can only be addressed by the acquisition of reliable oxylipin signatures through “oxylipin profiling”, and by the targeted modification of the abundance of the downstream enzymes, which are dedicated to individual oxylipin biosynthetic branches.

3.2 Enzymes downstream of LOXs: the CYP74 group

Among the enzymes of the oxylipin pathway able to utilize 9- and 13-LOX derivatives are AOS, HPL and DES, all of which belong to the CYP74 subfamily of P450 cytochromes. Compared to the typical P450 mono-oxygenases, CYP74 subfamily members lack oxygenase activity and utilize hydroperoxides both as oxygen donors and substrates (Itoh and Howe 2001; Song et al., 1993). Reactions catalyzed by CYP74s lead to the formation of JAs (*via* AOS), short chain ω-oxo fatty acids and volatile aldehydes (*via* HPL) and divinyl ethers (*via* DES) (Fig. 3) (Grechkin 2002). The members of the family can be grouped further on the basis of sequence similarity and substrate specificity: AOSs

(CYP74A) and some HPLs (CYP74B) have a higher affinity for 13-hydroperoxides, while other HPLs (CYP74C) and DESs (CYP74D) either preferentially utilize 9-hydroperoxides or lack any specificity. For a recent and comprehensive review of the biochemical properties and catalytic mechanisms of the CYP74 members, see Stumpe and Feussner (2006) (Stumpe and Feussner 2006).

3.2.1. *Allene oxide synthase (AOS)*

AOSs can metabolize both 9- and 13-LOX derivatives generated in chloroplasts (see §4.0). An unstable epoxide is generated from 9-HPOT, which spontaneously converts into 10-oPDA (Stumpe et al., 2006), the molecule responsible for tuber differentiation in potato (Kolomiets et al., 2001), or into α- and γ-ketols, the former of which act as stress signals during floral induction in *Lemna* (Yokoyama et al., 2000). The unstable product generated from 13-HPOT can be also enzymatically converted into JA, its conjugated derivatives and the bioactive precursor 12-oPDA (collectively termed jasmonates, JAs) or other α- and γ-ketols through a non- enzymatic conversion, as described earlier (Fig. 5) (Howe and Schilmiller 2002). For a review of JAs biosynthesis, see (Delker et al., 2006; Wasternack 2007). Recent studies indicate that the amino-conjugated form of JA, jasmonoyl-isoleucine (JA-Ile) is a bio-active form of JA which promotes the degradation of JASMONATE ZIM-domain (JAZ) transcriptional repressors through the activity of the E3 ubiquitin-ligase SCF^{COI1}. JA-Ile is presumed to be perceived at the level of the SCF^{COI1}-JAZ1 complex (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007) in the same way as is auxin on the SCF^{TIR1}-AUX/IAA receptor complex (Dharmasiri et al., 2005; Kepinski and Leyser 2005).

JAs were initially implicated in the plant response to wounding and insect attack (de Bruxelles and Roberts 2001; Stintzi et al., 2001; Thaler et al., 2002; Thaler et al., 2004; Thomma et al., 2001; Walling 2000), although it was recognized that they also have a role in the plant's primary metabolism and reproduction (Feys et al., 1994; van der Fits and Memelink 2000). Later, it became clear that JA is also involved in the defense response against microorganisms, specifically as a transcriptional regulator of pathogenesis-related genes (*e.g.* proteinase inhibitor - *PIN* genes), and, in conjunction with ET, as a mediator of ISR (Wasternack 2007). JAs have been ascribed a role in early signaling for SAR, in which they act upstream of SA in systemic leaves (Truman et al., 2007), and as essential components of the systemic wound signal [for review, see (Schilmiller and Howe 2005)].

They are also probably involved in the recently described wound-induced protection against pathogens (Francia et al., 2007). For a comprehensive review on the signaling networks involving JAs, see (Balbi and Devoto 2008).

Several lines of evidence support the key role played by AOS in JA production: indeed, knock-out *A. thaliana* mutants have shown that AOS activity is essential for JAs production, and their down-regulation induces a severe phenotype both following wounding, but also in the course of normal development (Park et al., 2002; von Malek et al., 2002). On the other hand, AOS-over-expressing plants show increased levels of JA only upon wounding, and reveal the close dependence of this pathway on the upstream LOXs and PLs, which regulate the basal level of JAs in plants by limiting substrate availability (Laudert et al., 2000). The inter-dependent and coordinated metabolism of AOS and LOXs has been demonstrated *via* transgenic manipulation of gene transcription, as in the following examples:

- the over-expression of an *A. thaliana* 13-AOS in both tobacco and *A. thaliana* is associated with high levels of JAs upon wounding, but not in unwounded tissues, within which upstream LOX activity is absent (Schaller 2001)
- the constitutive expression in tobacco of a flax 13-AOS lacking the characteristic chloroplast transit peptide results in increased JA synthesis only upon wounding, indicating the presence of a strictly wound-activated JA biosynthetic pathway not only in the chloroplast but also in the cytosol (Wang et al., 1999)

These data suggest that changes in JA content depend on the spatial and temporal expression of the genes responsible for its synthesis, on their compartmentalization and on the availability of precursors. Indications that JAs are necessary for resistance to a plethora of pathogens are numerous. Several mutants compromised in their synthesis, perception or downstream signaling are generally less resistant to pests (for a comprehensive and updated list, see Balbi et al, 2008). Successful gain-of-function approaches are less well documented, possibly because of limitations in the availability of appropriate substrates as described above. However, some examples of constitutive over-expression have been published – these include a pathogen-inducible AOS isoform and a jasmonate carboxyl methyl transferase (JMT, forming MeJA from JA), which confer,

respectively, resistance to *Magnaporthe grisea* in rice (Mei et al., 2006) and *Botrytis cinerea* in *A. thaliana* (Seo et al., 2001).

3.2.2. *Hydroperoxide lyase (HPL)*

Several products of this branch of the oxylipin pathway were identified long before the enzymes responsible for their formation were, and were described collectively as the ”green-leaf odor”, “green-leaf volatiles” (GLVs) or “leaf aldehydes” (Hatanaka and Harada 1973). HPLs catalyze the cleavage of hydroperoxy fatty acids between the conjugated diene and the carbon bearing the hydroperoxy group to generate aldehydes and aldoacids (Fig. 6) which spontaneously convert into several active isoforms (Grechkin 1998). For example, the initial aldoacid products derived from 13-HPOT can be metabolized into (10*E*)-12-hydroxy-10-dodecenoic acid, and the aldehydes into (3*Z*)- and (2*E*)-hexenols through the activity of an alcohol dehydrogenase (Grechkin et al., 1990). Other fates for 13-HPL derivatives can be a non-enzymatic oxidation (Noordermeer et al., 2000; Schneider et al., 2001) or an enzymatic conversion by a LOX or a POX (Gardner and Grove 1998). HPLs which selectively utilize the 9-LOX derivatives as substrates are able to form C9 compounds such as 9-oxo nonanoic acid and (3*Z*,6*Z*)- and (2*E*,6*Z*)-nonadienal aldehydes (Howe and Schilmiller 2002). The isomerization and oxidation of (9*Z*)-12-oxo-9-dodecenoic acid leads to the formation of traumatic acid (trans-2-dodecenedioic acid), a known growth factor, sometimes thought of as a “flowering hormone”. The traumatin aldehydes [(10*E*)-12-oxo-10-dodecenoic acid and (9*Z*)-12-oxo-9-dodecenoic acid], which are produced in equimolar ratio during the same processes as is traumatic acid, are also considered to act as growth hormones (Grechkin 1998). Their aldehyde group has been correlated with mitotic progression in dividing root cells of *Pisum sativum* (Ivanov et al., 2001). Aldehydes produced by 13-HPL activity are volatile, and include both hexenals (leaf aldehydes) and hexenols (leaf alcohols), both occurring in either the (3*Z*)- and (2*E*)-configuration). All these compounds are responsible for the characteristic smell of freshly mown grass. C9 aldehydes derived from 9-HPOT or 9-HPOD contribute to the characteristic smell and taste of several alimentary products (Grechkin 2002).

HPL derivatives are associated with the defense responses of various plants, because:

- they are able to induce defense-related genes in *A. thaliana* (Bate and Rothstein 1998; Kishimoto et al., 2005, 2006a, c)
- they are implicated in the defense response of *Phaseolus* against *Pseudomonas syringae* (Croft et al., 1993)
- they possess anti-microbial effects, thanks to the growth-inhibiting properties of aldehydes towards phytopathogenic fungi (Kishimoto et al., 2006b; Matsui et al., 2006; Prost et al., 2005; Vaughn and Gardner 1993). Volatile aldehydes can bind covalently to proteins and nucleic acids thus inhibiting their functionality (Golzer et al., 1996; Zidek et al., 1997); this makes them cytostatic and genotoxic, in mammals too (Esterbauer et al., 1991)
- HPL-encoding genes are rapidly expressed following wounding (Bate and Rothstein 1998; Howe et al., 2000). A specific 13-HPL is constitutively expressed in leaves and flowers of potato, and its role in the defense response against green peach aphids (*Myzus persicae*) has been demonstrated *via* an antisense-based transgenic strategy (Vancanneyt et al., 2001). Furthermore, in *A. thaliana* plants, whose GLV profile was altered both by over-expression or silencing of a HPL isoform, the level of resistance to the necrotrophic fungus *Botrytis cinerea* was proportional to GLV abundance; as was the plants' resistance to herbivory, mediated by a parasitoid wasp in a tripartite interaction (Shiojiri et al., 2006).

3.2.3. *Divinyl ether synthase (DES)*

Hydroperoxy fatty acids can be converted by DESs into conjugated ethers of PUFAs, called divinyl ethers (DVEs). These compounds possess an oxygen bridge within their aliphatic chain (Fig. 7). The taxonomic distribution of the DVE biosynthetic pathway is probably uneven, and is only properly documented for solanaceous plants. The origin of the characteristic ether group oxygen in these molecules has been the focus of long and detailed studies, because understanding it was felt to be important for revealing the catalytic mechanism of DESs. Early experiments using ^{18}O-labelled 9-hydroperoxides suggested that its origin was from the aqueous solvent (Galliard et al., 1975). However, later work established that the oxygen's origin is from within the substrate itself, thus confirming the peculiarity of CYP74Ds in utilizing hydroperoxides simultaneously as both oxygen donors and substrates (Grechkin et al., 1997).

While several AOS and HPL isoenzymes, which metabolize both 9- and 13-LOX derivatives, have been characterized, the DESs are more selective for 9-hydroperoxides (Itoh and Howe 2001). In tomato grown under standard conditions, DES mRNA expression is limited to non-photosynthetic organs. This suggests a stricter cooperation with 9-LOXs than with 13-LOXs, since 9-LOXs have the same preferential organ distribution [(Itoh and Howe 2001); see also §4.0). Solanaceous DESs can convert 9-hydroperoxides into colneleic acid (CA) and colnelenic acid (CnA), starting from, respectively, LA and LnA, but have no affinity for 13-LOX derivatives (Fammartino et al., 2007; Galliard et al., 1975; Itoh and Howe 2001). The only documented exceptions concern non-solanaceous species: for example, 13-DES activity present in garlic bulbs and *Ranunculus acris* leads to the production of the DVEs etheroleic and etherolenic acid (Fig. 7) (Grechkin et al., 1995; Hamberg 1998, 2002; Stumpe et al., 2008).

The DES branch of the oxylipin pathway is the only one known at present which is not induced upon wounding, since the HPL- and AOS-dependent routes are very much associated with the response to mechanical stress (Matsui 2006; Wasternack et al., 2006). Instead, DES transcription and activity are strongly induced during pathogen attack (Stumpe et al., 2001). Indications for a role of DVEs in plant defense have also emerged from oxylipin profiling of inoculated tissues from solanaceous plants (Fammartino et al., 2007; Göbel et al., 2002; Weber et al., 1999), where CA and CnA have both been found to accumulate to significant levels. CA and CnA are small molecules, which *in vitro* can suppress fungal growth and spore germination at low concentrations (Prost et al., 2005). This, together with their inducibility by pathogens, is why they are commonly considered as representing volatile phytoalexins (Weber et al., 1999). As for the *in vivo* role of CA and CnA in resistance, the bulk of evidence has been obtained from potato and tobacco.

In potato, the fully compatible interaction with *Phytophthora infestans* and the non-host interaction with the non-adapted bacterial pathogen *Pseudomonas syringae* pv. *maculicola* have been compared in wild-type plants. Oxylipin profiling has revealed that CA and CnA accumulate in both pathosystems, although more intensely and earlier in the incompatible one (Göbel et al., 2002; Weber et al., 1999). However, transgenic plants silenced for a pathogen-inducible 9-DES do not show any higher level of susceptibility to late blight disease than wild-type plants, in spite of their accumulation of significantly less DVEs upon infection (Eschen-Lippold et al., 2007). This has been interpreted to mean that

DVEs do not contribute to the eventually unsuccessful containment of the pathogen in a compatible interaction. Unfortunately no data are yet available regarding the response of DES-silenced plants to infection with the non-adapted bacterial pathogen. The situation is somewhat different for the race-cultivar specific interaction between tobacco and *Phytophthora parasitica* var. *nicotianae*. Expression of the pathogen-inducible gene for the 9-LOX *NtLOX1* was silenced, and this compromised the resistance to infection by the avirulent race 0 of the pathogen (Rancé et al., 1998). Oxylipin profiling of wild-type and transgenic roots has confirmed the involvement of a 9-LOX pathway in response to attempted infection, and revealed a prominent role for DVEs, whose production was indeed compromised in roots of plants not expressing *NtLOX1* (Fammartino et al., 2007). The lack of HPL and AOS derivatives in wild-type and transgenic roots upon infection suggests that these alternative branches of the oxylipin network are unlikely to be critical in this pathosystem. On the other hand, the pronounced accumulation (although slower than for CA and CnA) of 9-hydroxy-octadecadienoic acid (9-HOD) in infected wild-type but not transgenic roots, emphasizes the relevance of the 9-LOX pathway in this system (Fammartino et al., 2007). Further studies are clearly needed to discriminate the relative contribution of DVEs and 9-HOD to resistance to *P. parasitica*, particularly because 9-HOD is also fungitoxic (Cowley and Walters 2005; Prost et al., 2005).

4.0. *Cellular localization of the oxylipin pathway enzymes*

The tissue and intracellular localization of the various enzymes cooperating with one another in the oxylipin pathway can be informative regarding their physiological role. Almost all plant organs (cotyledons, seeds, fruits, roots and leaves) contain isoforms of LOX, both in the cytosol and in the sub-cellular compartments (Bell et al., 1995; Farmaki et al., 2007; Feussner and Kindl 1992; Matsui et al., 1992; Tranbarger et al., 1991; Vernooy-Gerritsen et al., 1984; Wardale and Lambert 1980). Soluble LOXs are present in the cytosol and vacuoles; in cotyledons, LOX aggregates are detectable in microsomal membranes (Feussner and Kindl 1994), in the plasma membrane (Nellen et al., 1995) and in lipid bodies (Feussner and Kindl 1992). In spinach, barley and *A. thaliana*, LOXs are concentrated in the chloroplast membranes (Bell et al., 1995; Blée and Joyard 1996; Feussner et al., 1995; Vick and Zimmerman 1987). If, as likely is the case, a precise oxylipin signature were determined by a combination of specific localization and differential

expression of the various LOX isoforms, a high degree of specialization for the members of this family would be expected. The evidence is that 13-LOXs are rare in the cytosol but common in the chloroplasts, whereas 9-LOXs are restricted to the cytoplasm (Feussner and Wasternack 2002). This is consistent with the observation that 13-LOX metabolism is more active than 9-LOX in photosynthetically active organs (see also §3.1) (Itoh and Howe 2001).

Much less is known regarding the sub-cellular localization of the CYP74 enzymes acting downstream of LOXs than that of the LOXs themselves. Most of the AOSs and HPLs characterized to date appear to be compartmentalized. Several CYP74 cDNAs bear a predicted plastid transit peptide sequence (Howe et al., 2000), and the corresponding proteins utilize specific import proteins to cross the double membrane of the chloroplasts (Froehlich et al., 2001), where they can be immuno-localized (Maucher et al., 2000). Notably, 13-HPLs are concentrated in the inter-membrane space, while 13-AOSs remain in the inner compartment. In a detailed study of potato leaves using a combination of confocal microscopy of GFP-tagged proteins, chloroplast fractionation, western blotting and immuno-detection by electron microscopy, several LOX pathway enzymes were successfully localized within the chloroplasts. While the 13-LOX isoforms were found in both the stroma and the thylakoids, both AOS and HPL were almost exclusively bound strongly to the thylakoid membranes. AOC was weakly associated with the thylakoid membrane and was also detected in the stroma. Moreover, AOS and HPL were differentially distributed in thylakoids, with HPL concentrated at the stromal face. In addition to their differential expression pattern, this spatial segregation may well underlie the regulation of metabolic fluxes through the AOS or HPL branches of the 13-LOX pathway (Farmaki et al., 2007). With respect to the 9-HPL metabolism, one documented case (in almond seed) found 9-HPL protein to be associated with lipid bodies in the cytoplasm or with the endomembrane system (Mita et al., 2007), especially during fungal infection of immature seeds. [Note that C9 aldehydes are produced during seed colonization by *Aspergillus carbonarius* (Mita et al., 2005)]. In *Medicago truncatula*, a 9/13-HPL isoform was found in lipid bodies, while a 13-HPL was found in the plastids (De Domenico et al., 2007). Finally, a 9-AOS from potato has been localized to the outer membrane of the plastid envelope (Stumpe et al., 2006).

There are some indications for the localization of DES in the microsomal fraction of potato leaf and in garlic bulbs (Fahlstadius and Hamberg 1990; Grechkin and Hamberg 1996). However, the lack of any organelle-targeting sequence rather suggests a cytosolic location, although the absence of an obvious transit peptide does not necessarily imply non-targeting, as exemplified by barley AOSs (Maucher et al., 2000). A cytosolic location would be compatible with the enrichment for 9-LOXs associated with the same compartment, and with the preference of DESs for 9-hydroperoxides, as described in §3.2.3. Thus, the cytosol is a likely candidate location for the synthesis of 9-DVEs (Feussner and Wasternack 2002). Supportive of this is a body of evidence derived from confocal microscopy, which shows that in tobacco leaves expressing 9-DES:YFP and 9-LOX:CFP fusion proteins, the two enzymes co-localize in the cytosol (Fammartino et al., 2007).

The overall picture for the sub-cellular localization of 9- and 13-LOXs, 13-AOSs, 9- and 13-HPLs, and 9-DESs, is one in which the 13-hydroperoxide-converting enzymes are located in the inner membrane of the chloroplast envelope (Fig. 8), while the 9-hydroperoxide-metabolizing CYP74s are in the outer membrane or the cytosol (Feussner and Wasternack 2002).

5.0. *Interactions between oxylipins and other defense-related pathways*

"Cross-talk" is a term widely used to describe generic, direct or indirect influences between signaling pathways. The term is popular because it is flexible enough to include both positive and negative signaling, modulation of gene expression patterns and feedback. It is also applied to describe specific interactions between components of more than one pathway. Both these meanings imply the balancing of signal specificity and integration (Mundy et al., 2006). The term is used here in the sense of any shared node in signaling networks generated by different stimuli. This includes distinct pathways achieving the same end, or those interacting and affecting one another's outcome in either an additive or a negatively regulatory fashion, or competing for a common target (Knight and Knight 2001). Conversely, specificity implies that a given part of a signaling pathway directs between two or more possible outcomes, thereby linking a particular stimulus exclusively to a particular end response. Opportunities for both integration and specificity occur within most pathways, and are largely responsible for the complexity of defense signaling

(Kunkel and Brooks 2002). For clarity, we concentrate here on the oxylipin pathway and other pathways or molecules directly relevant to defense. Signaling pathways leading to, for example, wound-induced JA biosynthesis are not considered here, unless relevant in the context of cross-talk; for a wide review of this topic, see Balbi et al, (2008) (Balbi and Devoto 2008).

Generally speaking, JA is responsible for the positive stimulation of other oxylipin branches (Wasternack 2007). Furthermore, cross-talk occurs among various oxylipins branches, with the effect that the silencing of one branch enhances activity in another. For example, in *Nicotiana attenuata,* the loss of a 13-HPL leads to over-production of JA in response to wounding, and the silencing of a 13-AOS to enhanced GLV synthesis (Halitschke et al., 2004). When transgenic potato, silenced with respect to 9-LOX metabolism, was challenged by a non-host pathogen, there was no evidence for heightened susceptibility to the pathogen, despite the lower abundance of 9-LOX metabolites. In compensation, the 13-LOX pathway was over-stimulated, and the resulting phenotype was interpreted as possibly deriving from the antimicrobial and signaling capacity of the upregulated 13-LOX metabolites. (Göbel et al., 2003). In these cases, substrate availability and supply may be regulating the balance between the metabolic flows. In tobacco, preliminary data indicate that the activity of the 9-DES biosynthetic pathway is enhanced by JA and ethylene (ET), and inhibited by SA (Fammartino, Cardinale et al, unpublished data).

Actually, a greater deal is known about cross-talk between JA and other hormones or signal molecules, which include SA and ET. Together with JA, these act to modulate the plant defense response (Beckers and Spoel 2006). SA is an indispensable signal for the initiation of SAR (Gruner et al., 2003; Sticher et al., 1997), while ET is an important component both in the responses to mechanical damage, herbivory and pathogen attack, and in developmental processes such as senescence and fruit ripening (Adie et al., 2007; Broekaert et al., 2006). JA, which is itself an oxylipin (see §3.2.1.), is involved in the wound and pathogen responses of plants (O'Donnell et al., 2003; van Wees et al., 2000), and has recently been shown to also participate in SAR onset (Truman et al., 2007). A generic signaling scheme has been proposed (although several exceptions have been described) in which HR in response to biotrophic infection requires the production of SA, whereas the response to necrotrophs requires JA and ET (Feys and Parker 2000). Roles for ET, SA,

and JA have also been proposed in the regulation of susceptible responses (Francia et al., 2007; Greenberg et al., 2000; Pilloff et al., 2002).

Both targeted marker expression analyses and transcriptomic approaches have revealed the extent of specificity and overlap in the responses to these three signal molecules, and of cross-talk between their signaling pathways. The use of mutants, particularly of *A. thaliana*, expressing enhanced susceptibility or resistance to pathogens, or insensitivity to exogenously applied SA, JA or ET, has allowed the first steps to be made towards identifying the signaling networks involving these molecules (Jalali et al., 2006). SA is thought to act antagonistically to ET and JA (both at the level of synthesis and downstream signaling) (Wasternack et al., 2006). The latter act synergistically with one another to coordinate the expression of defense-related genes in ISR (Schenk et al., 2000; Xu et al., 1994). Although SA is commonly regarded as antagonistic in JA/ET-dependent pathways, in tomato ET is thought to positively modulate certain SA-dependent responses (Diaz et al., 2002); while in *A. thaliana,* low doses of JAs and SA have been shown to act synergistically (Mur et al., 2006). Opportunities for cross-pathway dialogue have been elegantly represented in the 'tunable dial' model of Reymond and Farmer (Reymond and Farmer 1998), allowing for both synergistic and antagonistic relationships between signal molecules, depending on their relative concentrations. The differential activation of JA- and SA-signaling pathways, in terms of early *vs* late induction, local *vs* systemic action, and high *vs* low biosynthetic rates may provide plants with an adaptive defense mechanism.

The network nodes - that is, the molecular determinants of cross-talk between the SA, ET and JA pathway – are now being identified. In *A. thaliana*, these comprise several transcription (co)factors, including NPR1 (an essential mediator of SA) and WRKY53, WRKY62 and WRKY70 (Fig. 9) (Balbi and Devoto 2008; Eulgem and Somssich 2007). The MAPK isoform AtMPK4 is a further cross-talk player between the SA and JA/ET pathways (Andreasson et al., 2005; Brodersen et al., 2006; Petersen et al., 2000). The unusual antagonism of *A. thaliana* AtERF1 and AtMYC2 illustrates rather well the intricacy of the network underlying the transcriptional response to stress: the former positively regulates defense-related, ET-induced genes and inhibits a subset of wound-related, JA-induced genes; while the second does the opposite (Lorenzo et al., 2004). In tobacco, CDPK2 (a Ca^{2+}-dependent protein kinase) mediates, *via* ET, the inhibition of MAPK

activation (isoforms SIPK and WIPK) (Ludwig et al., 2005), a process which is required for wound-induced JA production (Gomi et al., 2005; Seo et al., 2007).

The phytohormones ET and JA respond to a wide range of environmental stimuli and are involved in a range of developmental and stress responses. In addition to well-conserved core responses, some specificity in terms of the final outcome is derived from the integration of all signaling and hormonal pathways activated under a particular environmental stimulus (Thomma et al., 2001). Calcium ions (Ca^{2+}), ROS and nitric oxide (NO) are ubiquitously present at many signaling cross-roads, and are often viewed as potential connection points between pathways (Fujita et al., 2006). The latter two are both indicators of physiological imbalance and inducers of defense responses (Garcia-Brugger et al., 2006; Hong et al., 2008; Kotchoni and Gachomo 2006). Typically, following the successful recognition of a pathogen, an oxidative burst is generated, leading to ROS [predominantly superoxide (O_2^-) and hydrogen peroxide] production . Several lines of evidence prove that ROS are needed for mounting the defense response, and in particular for initiating HR (Torres et al., 2006). Moreover, they are known to positively interact with SA in establishing SAR (Durrant and Dong 2004), and require a functional JA signaling pathway to be generated upon wounding (Orozco-Cardenas et al., 2001). NO is thought to act synergistically with ROS in the stimulation of apoptosis in the cells surrounding a pathogen invasion site (Delledonne et al., 1998; Delledonne et al., 2001; Lamotte et al., 2004). Some instances where the ROS and NO signaling pathways positively modulate one another have also been reported (Tada et al., 2004; Zeier et al., 2004). The rapid accumulation of free cytosolic Ca^{2+} represents a particularly common event, and the oxidative burst and HR are associated with enhanced Ca^{2+} concentrations in the cytosol (Blume et al., 2000; Grant et al., 2000; Lecourieux et al., 2006). The contemporaneous presence of ROS, NO and Ca^{2+} with oxylipins has been demonstrated in several plant-microbe interactions, and also in the perception process of both very general and more specific elicitors (Blanco et al., 2008; Garcia-Brugger et al., 2006; Tyler 2002). An example is provided by cryptogein perception in tobacco, where ROS-induced lipid peroxidation contributes to a small extent to programmed cell death, a process which relies mainly on LOX-produced 9-hydroperoxides (Lecourieux et al., 2006; Montillet et al., 2005).

The possibility that oxylipins cross-talk with other pathways through these mediators cannot be ruled out. So far, however, with the sole exception of the connection between

JA and NO synthesis (Lamotte et al., 2004) or Ca^{2+} influx (Bonaventure et al., 2007; Fisahn et al., 2004), no cause-effect links have been established as yet between other branches of the oxylipin metabolism and ROS, NO and Ca^{2+}. The identification of common transducers of these pathways would help to understand how cross-talk occurs. However, it is clear that transducers such as MAPKs, their inactivating phosphatases (Schweighofer et al., 2007) and CDPKs are necessary for JA production, and represent an integration point for a number of distinct environmental stimuli. Thus they become clear candidates as agents of cross-talk between signaling pathways. The data, which have been accumulating on defense response signaling downstream of non-self perception, point to a complex interplay among pathways. Interactions may be positive or negative, depending on the spatial and temporal generation of the mediators, on the intensity of signal flow in the various pathways and, in the end, on the nature of the perceived stimulus.

6.0. *Future scope and conclusions*

This overview was intended to provide a window on the complexity of the contribution of the oxylipins to plant defense against pathogens, with an emphasis on discoveries flowing from functional genetics approaches. Previous reports have described the potential of various oxylipins in plant defense (Blée 1998, 2002), their activity as phytoalexin-like compounds (Prost et al., 2005) and their role in the signaling processes required for the induction of wound- and pathogen-related defense responses (Balbi and Devoto 2008; Wasternack et al., 2006). The involvement of the oxylipin-producing LOX enzymes in HR, which conditions immunity both in host and non-host incompatible interactions, is well documented (Fammartino et al., 2007; Mène-Saffrané et al., 2003; Montillet et al., 2005; Rancé et al., 1998; Rusterucci et al., 1999). However much remains to be discovered before the full contribution of the oxylipins to adaptation to an ever-changing environment can be resolved. In the context of synthesis, post-transcriptional modification (in particular, but not limited to, phosphorylation/ dephosphorylation), which can affect enzyme stability, activity and/or localization, is likely to become an area of more intensive research activity. Another likely fruitful research direction relates to the perception and signal transduction of the oxylipins, and their interference with other pathways. The great diversity among the oxylipins as a group has enormous potential in this area, as it allows for amplification of the signal generated by incoming stress stimuli, diversification and fine-tuning of the anti-

microbial metabolites produced in response to them, along with many opportunities for cross-talk within the plant defense network.

7.0. *References*

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Oxylipin	Short Name	Reference
2(*R*,*S*)-Hydroxy-9(*Z*)-octadecenoic acid	2-HOE	1
2(*R*)-Hydroxy-9(*Z*),12(*Z*),15(*Z*)-octadecatrienoic acid	2-HOT	1
8(*Z*),11(*Z*),14(*Z*)-Heptadecatrienal	17:3-al	1
9(*S*)-Hydroperoxy-10(*E*),12(*Z*),15(*Z*)-octadecatrienoic acid	9-HPOT	2, 3
9(*S*)-Hydroperoxy-10(*E*),12(*Z*)-octadecadienoic acid	9-HPOD	2, 3
11(*S*)-Hydroperoxy-7(*Z*),9(*E*),13(*Z*)-hexadecatrienoic acid	11-HPHT	2, 3
13(*S*)-Hydroperoxy-6(*Z*),9(*Z*),11(*E*)-octadecatrienoic acid	13-HPOTω6	2, 3
13(*S*)-Hydroperoxy-9(*Z*),11(*E*),15(*Z*)-octadecatrienoic acid	13-HPOT	2, 3
13(*S*)-Hydroperoxy-9(*Z*),11(*E*)-octadecadienoic acid	13-HPOD	2, 3
9(*S*)-Hydroxy-10(*E*),12(*Z*),15(*Z*)-octadecatrienoic acid	9-HOT	2, 3
9(*S*)-Hydroxy-10(*E*),12(*Z*)-octadecadienoic acid	9-HOD	2, 3
11(*S*)-Hydroxy-7(*Z*),9(*E*),13(*Z*)-hexadecatrienoic acid	11-HHT	2, 3
13(*S*)-Hydroxy-6(*Z*),9(*Z*),11(*E*)-octadecatrienoic acid	13-HOTω6	2, 3
13(*S*)-Hydroxy-9(*Z*),11(*E*),15(*Z*)-octadecatrienoic acid	13-HOT	2, 3
13(*S*)-Hydroxy-9(*Z*),11(*E*)-octadecadienoic acid	13-HOD	2, 3
9-Keto-10(*E*),12(*Z*),15(*Z*)-octadecatrienoic acid	9-KOT	2, 3
9-Keto-10(*E*),12(*Z*)-octadecadienoic acid	9-KOD	2, 3
13-Keto-9(*Z*),11(*E*),15(*Z*)-octadecatrienoic acid	13-KOT	2, 3
13-Keto-9(*Z*),11(*E*)-octadecadienoic acid	13-KOD	2, 3
(±)-cis-9,10-Epoxyoctadecanoic acid	9,10-EO	4
9(*R*),10(*S*)-Epoxy-12(*Z*)-octadecenoic acid	9,10-EOE	4
12(*R*),13(*S*)-Epoxy-9(*Z*)-octadecenoic acid	12,13-EOE	4
(±)-threo-9,10-Dihydroxy-12(*Z*)-octadecenoic acid	9,10-DHOE	4
(±)-threo-12,13-Dihydroxy-9(*Z*)-octadecenoic acid	12,13-DHOE	4
9,10,16-Trihydroxyhexadecanoic acid (aleuritic acid)	9,10,16-THH	4
12-Oxo-10,15(*Z*)-phytodienoic acid	12-Oxo-PDA	5, 6
13-Hydroxy-12-keto-9(*Z*),15(*Z*)-octadecadienoic acid	12,13-KHOD	7
2(*E*)-Hexenal	2-Hexenal	–
3(*Z*)-Hexenal	3-Hexenal	4
2(*E*)-Nonenal	2-Nonenal	4
3(*Z*)-Nonenal	3-Nonenal	4
9-Oxononanoic acid	9-Oxo-C_9	4
12-Oxo-9(*Z*)-dodecenoic acid	12-Oxo-12:1(Z)	4
12-Oxo-10(*E*)-dodecenoic acid	12-Oxo-12:1(E)	4
1-Penten-3-ol	5:1-ol	–
Colnelenic acid	CLn	8
Colneleic acid	CL	8
ω5(*Z*)-Etherolenic acid	ω5(Z)-ELn	9
10(*S*),11(*S*)-Epoxy-9(*S*)-hydroxy-12(*Z*),15(*Z*)-octadecadienoate	9,10,11-EHOD	10
11(*S*),12(*S*)-Epoxy-13(*S*)-hydroxy-9(*Z*),15(*Z*)-octadecadienoate	11,12,13-EHOD	10
9(*S*),12(*S*),13(*S*)-Trihydroxy-10(*E*),15(*Z*)-octadecadienoic acid	9,12,13-THOD	10
9(*S*),12(*S*),13(*S*)-Trihydroxy-10(*E*)-octadecenoic acid	9,12,13-THOE	10
9(*S*),10(*S*),11(*R*)-Trihydroxy-12(*Z*)-octadecenoic acid	9,10,11-THOE	10
11(*R*),12(*S*),13(*S*)-Trihydroxy-9(*Z*)-octadecenoic acid	11,12,13-THOE	10

Table 1. Common abbreviations for the oxylipins and references to the relevant biosynthetic enzyme or chemical synthesis method. The references are as follows: 1: (Hamberg 1999); 2: (Gardner 1996); 3: (Lie Ken Jie and Pasha 1998); 4: (Gunstone et al., 1994); 5: (Baertschi et al., 1988); 6: (Hamberg and Fahlstadius 1990); 7: (Hamberg 1987);

8: (Galliard and Phillips 1972); 9: (Hamberg 1998); and 10: (Hamberg 1991). Modified from Prost et al. (2005).

Figure legends

Figure 1. Stereospecific activity of the lipoxygenases (LOXs). 9- and 13-LOXs act on an aliphatic chain (here α-linolenic acid, $18:3^{\Delta 9Z,12Z,15Z}$) to generate 9- and 13-hydroperoxides (9-/13-HPOT), depending on their specificity. Modified from Feussner and Wasternack (2002).

Figure 2. The structure of some oxylipins and their fatty acid precursors. Definitions and abbreviated names for the oxylipins as listed in Table 1. JA: jasmonic acid, α-DOX: α-dioxygenase, LOX: lipoxygenase, POX: peroxygenase, AOS: allene oxide synthase, HPL: hydroperoxide lyase, DES: divinyl ether synthase, EAS: epoxy alcohol synthase, PL: phospholipase. Modified from Prost et al. (2005).

Figure 3. Oxylipin biosynthetic pathways. 9- and 13-LOX pathways deriving from linoleic (LA, $18:2^{\Delta 9Z,12Z}$) or linolenic acids (LnA, $18:3^{\Delta 9Z,12Z,15Z}$), the major plant polyunsaturated fatty acids are illustrated. Abbreviations as in Figure 2. Branches initiated by CYP74 enzymes are indicated by red arrows. Modified from Blée (2002).

Figure 4. Stereospecific activity of lipoxygenases (LOXs). The models seek to understand the regiospecificity of the reaction mechanism at the catalytic site. Modified from Feussner and Wasternack (2002).

Figure 5. The allene oxide synthase (AOS) pathway. The figure shows (*left*) the metabolism of α-linolenic acid ($18:3^{\Delta 9Z,12Z,15Z}$) by the AOS branch of 9-LOX, and (*right*) 13-LOX pathways, leading to the synthesis of jasmonic acid and its derivatives. Abbreviations: lipoxygenase (LOX); allene oxide cyclase (AOC); allene oxide synthase (AOS); hydroperoxide lyase (HPL); 12-oxo-phytodienoic acid reductase (OPR); acyl-CoA oxidase (ACX); multifunctional protein (MFP); 3-ketoacyl-CoA thiolase (KAT). oPDA, oxo-phytodienoic acid; MeJA, methyl jasmonate; JA-ACC, conjugate with 1-aminocyclopropane-1-carboxylic acid; JA–Ile, conjugate with the amino acid isoleucine; 12-HSO4-JA, sulfonated derivative of the 12-hydroxy-jasmonic acid; 12-O-GlcJA, glucosylated derivative of the 12-hydroxy-jasmonic acid; JA-O-Glc, glucosylated derivative of JA (adapted from Itoh et al., 2002; Wasternack, 2007; Balbi and Devoto, 2008).

Figure 6. The conversion of 13-HPOT *via* hydroperoxide lyase (HPL) activity. Taken from Grechkin (2002)**.**

Figure 7. Divinyl ethers reported to date in plants. (A) colneleic acid (B) colnelenic acid (C) etheroleic acid (D) etherolenic acid. Taken from Grechkin (2002)**.**

Figure 8: Phyto-oxylipin cascades leading to defense responses in various sub-cellular compartments. Abbreviations as in figures 2 and 5. Metabolites (square boxes): PUFAs, poly-unsaturated fatty acids; GLVs, green leaf volatiles; oPDA, 12-oxo-phytodienoic acid; JAs, (+)-7-iso-jasmonic acid and its derivatives. Modified from Blée (2002) and Feussner and Wasternack (2002).

Figure 9. A working model for a regulatory network integrating WRKY-transcription factors under the control of salicylic acid (SA) and jasmonates (JAs) during defense responses. Green arrows indicate induction; blunt ends indicate repression. Modified from Balbi and Devoto (2008).

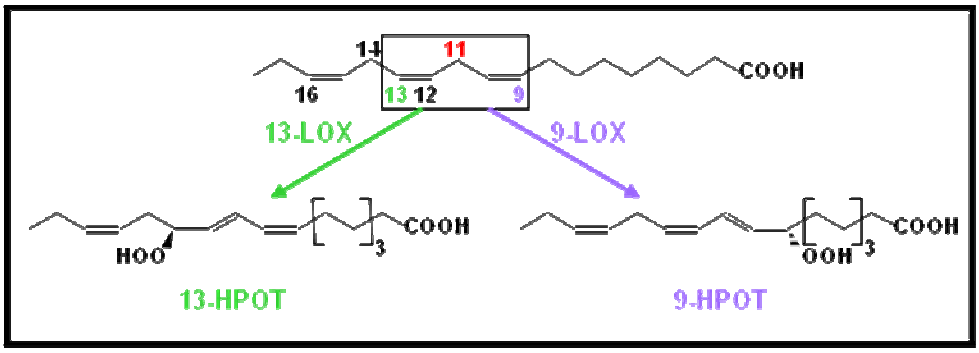

16
15
11
13
12
9
COOH
13-LOX
9-LOX
HOO
COOH
3
13-HPOT
OOH
COOH
3
9-HPOT

Figure 1

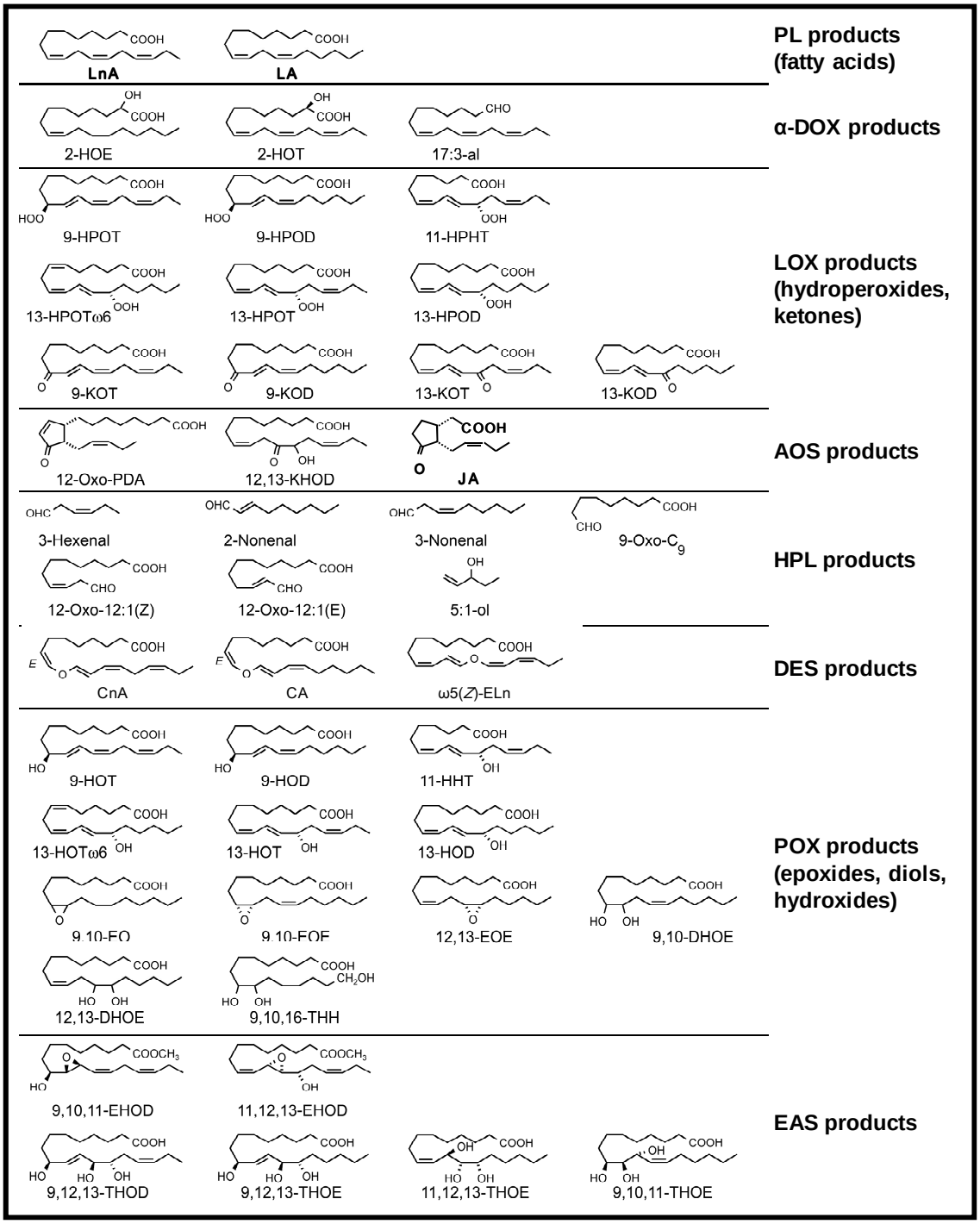
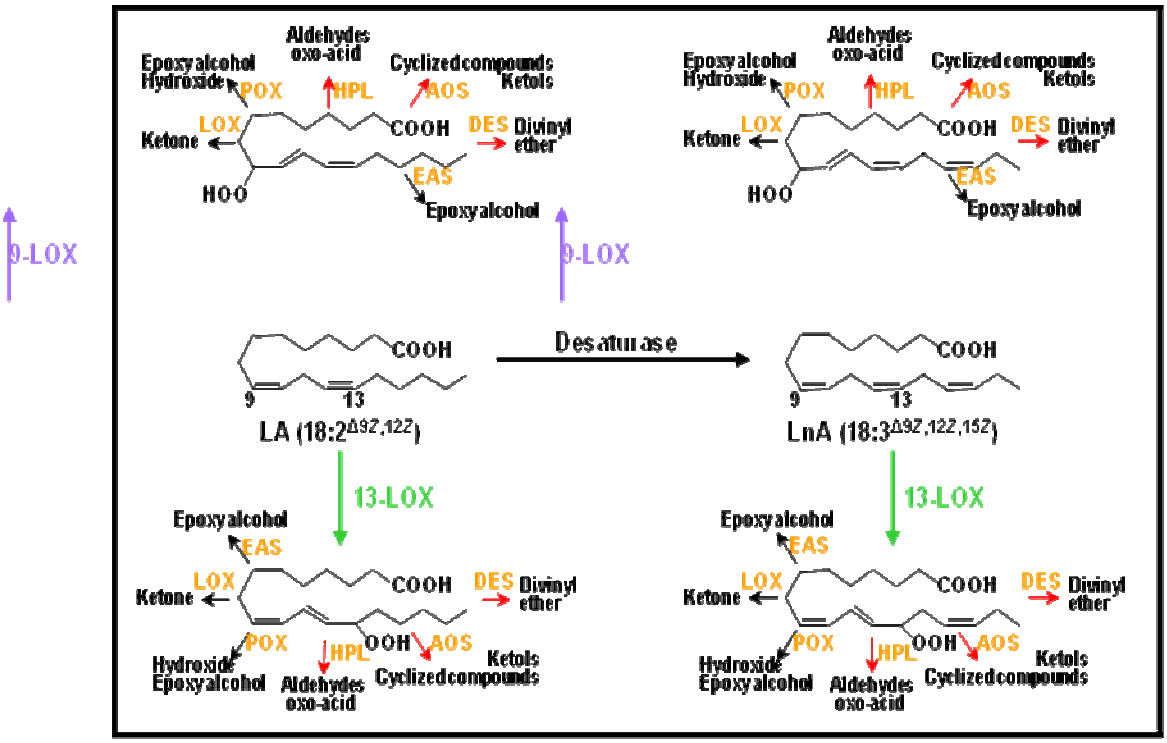

LnA
LA
PL products (fatty acids)
2-HOE
2-HOT
17:3-al
α-DOX products
9-HPOT
9-HPOD
11-HPHT
13-HPOTω6
13-HPOT
13-HPOD
9-KOT
9-KOD
13-KOT
13-KOD
LOX products (hydroperoxides, ketones)
12-Oxo-PDA
12,13-KHOD
JA
AOS products
3-Hexenal
2-Nonenal
3-Nonenal
9-Oxo-C9
12-Oxo-12:1(Z)
12-Oxo-12:1(E)
5:1-ol
HPL products
CnA
CA
ω5(Z)-ELn
DES products
9-HOT
9-HOD
11-HHT
13-HOTω6
13-HOT
13-HOD
9,10-EO
9,10-EOE
12,13-EOE
9,10-DHOE
12,13-DHOE
9,10,16-THH
POX products (epoxides, diols, hydroxides)
9,10,11-EHOD
11,12,13-EHOD
9,12,13-THOD
9,12,13-THOE
11,12,13-THOE
9,10,11-THOE
EAS products

Figure 2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Hydroxide
Aldehydes
oxo-acid
Cyclizedcompounds
Ketols
POX
HPL
AOS
LOX
Ketone
COOH
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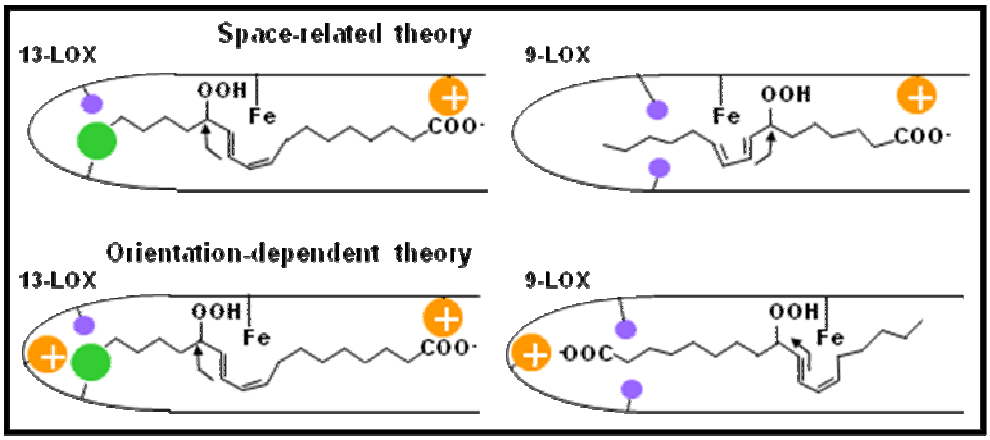
Divinyl
ether
HOO
EAS
Epoxyalcohol
9-LOX
9-LOX
Desaturase
9
13
LA (18:2Δ9Z,12Z)
LnA (18:3Δ9Z,12Z,15Z)
13-LOX
13-LOX
OOH
Hydroxide
Epoxyalcohol

Figure 3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OOH
Fe
COO-
9-LOX
Fe
OOH
COO-
Orientation-dependent theory
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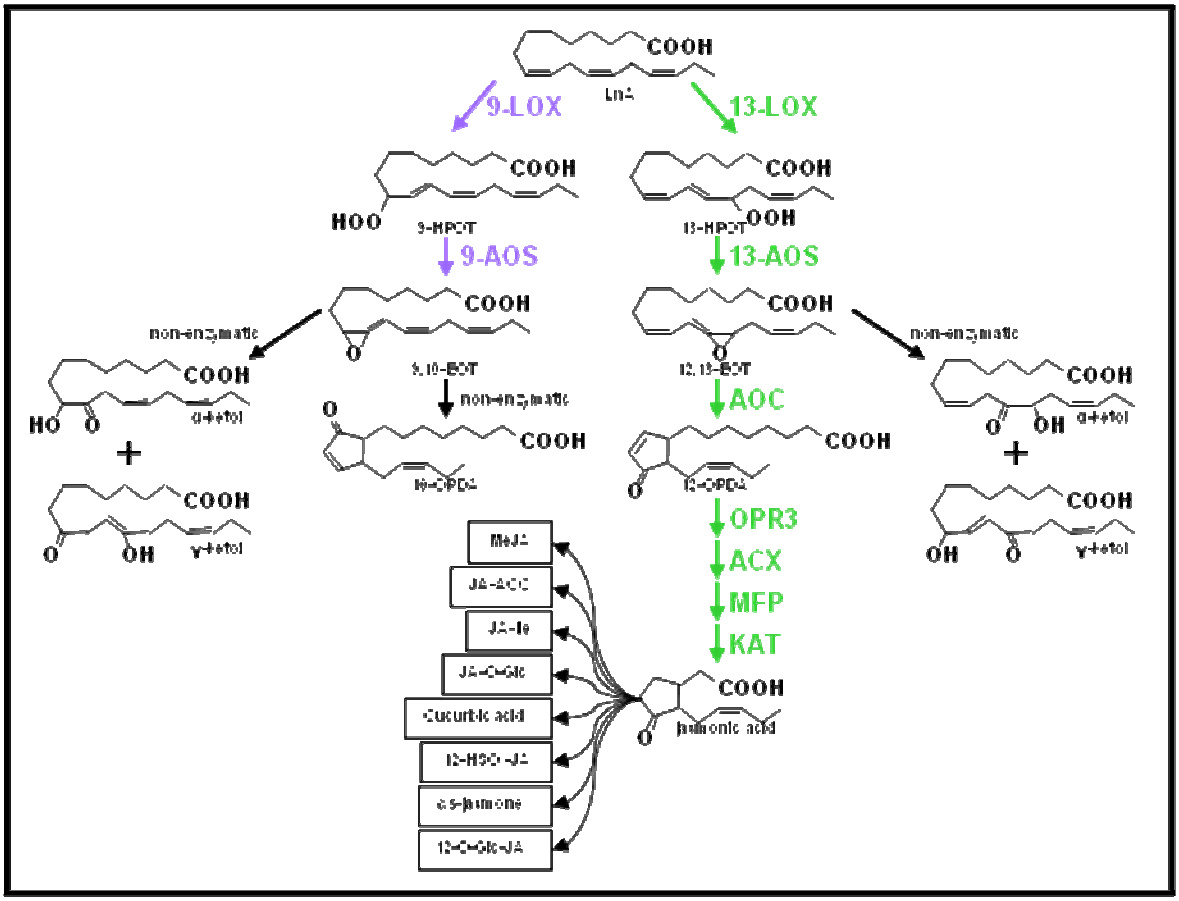
Fe
COO-
9-LOX
-OOC
OOH
Fe

Figure 4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Ln
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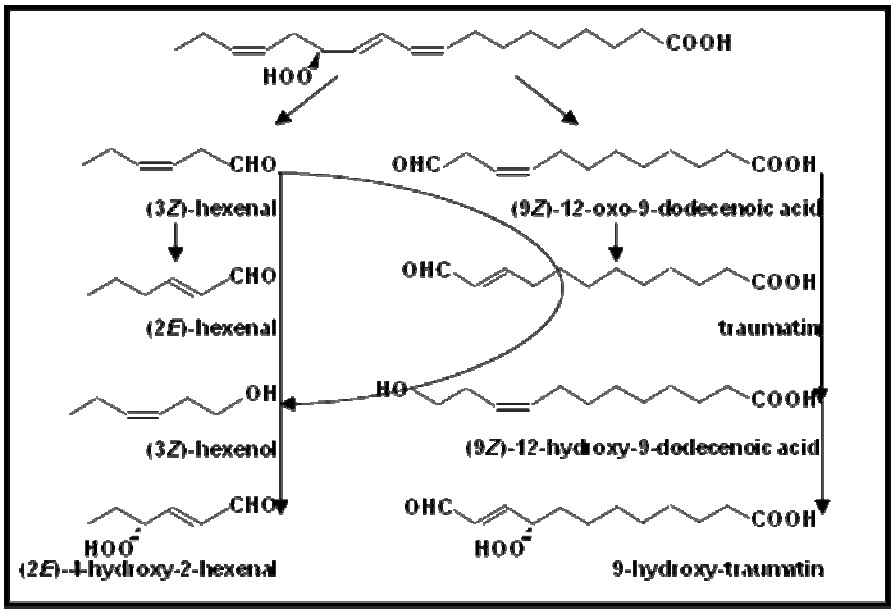
13-LOX
HOO
9-HPOT
13-HPOT
9-AOS
13-AOS
non-enzymatic
9,10-EOT
12,13-EOT
AOC
12-OPDA
OPR3
ACX
MFP
KAT
jasmonic acid
MeJA
JA-Ile
cucurbic acid
cis-jasmone

Figure 5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HOO
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OHC
COOH
(9Z)-12-oxo-9-dodecenoic acid
CHO
(2E)-hexenal
OHC
COOH
traumatin
OH
(3Z)-hexenol
HO
COOH
(9Z)-12-hydroxy-9-dodecenoic acid
CHO
HOO
(2E)-4-hydroxy-2-hexenal
OHC
COOH
HOO
9-hydroxy-traumatin

Figure 6

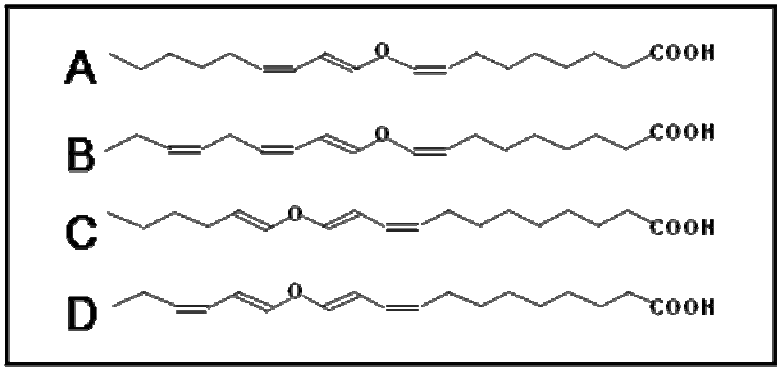

Figure 7

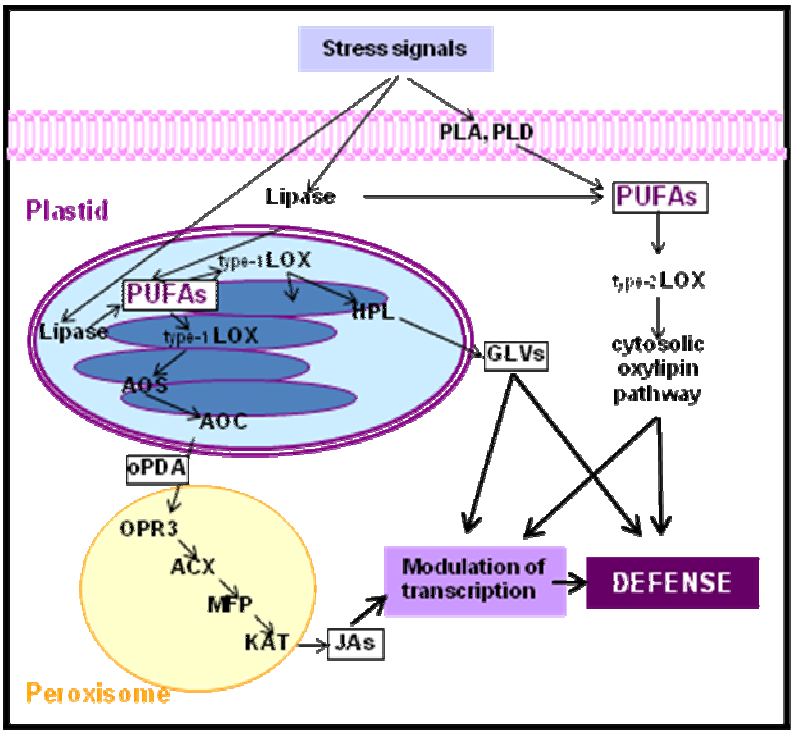
Stress signals
PLA, PLD
Plastid
Lipase
PUFAs
type-1 LOX
PUFAs
HPL
Lipase
type-1 LOX
GLVs
AOS
AOC
oPDA
type-2 LOX
cytosolic
oxylipin
pathway
OPR3
ACX
MFP
KAT
JAs
Modulation of
transcription
DEFENSE
Peroxisome

Figure 8

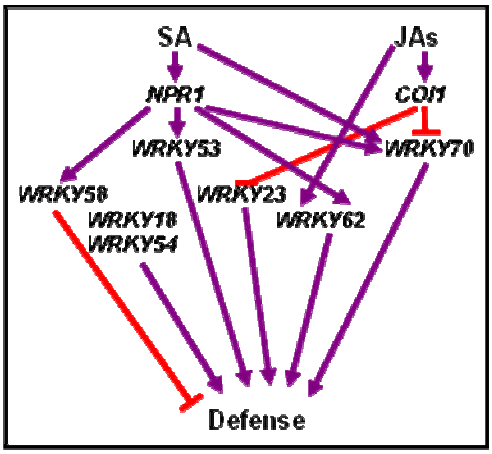
SA
JAs
NPR1
COI1
WRKY53
WRKY70
WRKY58
WRKY23
WRKY18
WRKY54
WRKY62
Defense

Figure 9