The Oxylipin Pathway in Arabidopsis

Authors: Creelman, Robert A., and Mulpuri, Rao

Source: The Arabidopsis Book, 2002(1)

Published By: The American Society of Plant Biologists

URL: https://doi.org/10.1199/tab.0012

First published on August 12, 2002: e0012. doi: 10.1199/tab.0012

The Oxylipin Pathway in *Arabidopsis*

Robert A. Creelman[a1] and Rao Mulpuri[b]

[a]Mendel Biotechnology, Incorporated, 21375 Cabot Blvd., Hayward, CA 94545
[b]Paradigm Genetics, Inc., 108 Alexander Drive, Research Triangle Park, NC 27709
[1]Corresponding author, email: bcreelman@mendelbio.com, phone: 510-259-6109, fax: 510-264-0254

ABSTRACT

Oxylipins are acyclic or cyclic oxidation products derived from the catabolism of fatty acids which regulate many defense and developmental pathways in plants. The dramatic increase in the volume of publications and reviews on these compounds since 1997 documents the increasing interest in this compound and its role in plants. Research on this topic has solidified our understanding of the chemistry and biosynthetic pathways for oxylipin production. However, more information is still needed on how free fatty acids are produced and the role of beta-oxidation in the biosynthetic pathway for oxylipins. It is also becoming apparent that oxylipin content and composition changes during growth and development and during pathogen or insect attack. Oxylipins such as jasmonic acid (JA) or 12-oxophytodienoic acid modulate the expression of numerous genes and influence specific aspects of plant growth, development and responses to abiotic and biotic stresses. Although oxylipins are believed to act alone, several examples were presented to illustrate that JA-induced responses are modulated by the type and the nature of crosstalk with other signaling molecules such as ethylene and salicylic acid. How oxylipins cause changes in gene expression and instigate a physiological response is becoming understood with the isolation of mutations in both positive and negative regulators in the jasmonate signaling pathway and the use of cDNA microarrays.

INTRODUCTION

Oxylipins are acyclic or cyclic oxidation products derived from the catabolism of fatty acids which regulate many defense and developmental pathways in plants. For example, jasmonic acid (JA), and its methyl ester (methyl jasmonate, MeJA) are linolenic acid (LA; 18:3) derived cyclopentanone based compounds of wide distribution in the plant kingdom. Early studies showed that exogenous JA or MeJA could promote senescence and act as a growth regulator. Subsequent research revealed that JA specifically alters gene expression and that wounding and elicitors could cause JA/MeJA accumulation in plants. These results implied a role for jasmonate in plant defense. Other research described roles for jasmonates in vegetative development and pollen viability. More recent studies have revealed that several compounds derived from octadecanoid or hexadecanoid fatty acid precursors function in plant growth and development.

This review will emphasize new information in this field since a recent review (Farmer et al., 1998) and will focus on studies utilizing *Arabidopsis thaliana* (although if needed information from other model plants will be included). In addition, this review will focus primarily on JA/MeJA (collectively called jasmonates in this review). The presence of mutants in fatty acid metabolism and the Arabidopsis Genome Initiative make *A. thaliana* the perfect tool to characterize the oxylipin pathway in plants. Other excellent reviews are available that cover oxylipins (in particular JA/MeJA) with respect to herbivory (Baldwin, 1999), disease (Dong, 1998), signaling (Farmer et al., 1998), biochemistry (Schaller, 2001; Howe and Schilmiller, 2002), and gene expression (Reymond and Farmer, 1998).

OXYLIPIN CHEMISTRY AND QUANTITATION

JA (Figure 1) contains two chiral centers located at C3 and C7 generating four possible stereoisomers, since either chiral center can have an R or S absolute configuration. Biosynthesis of JA yields the (+)-7-iso-JA stereoisomer having a 3R, 7S configuration. During extraction, in the presence of acids or bases, or in heated inlets during gas chromatographic analysis, (+)-7-iso-JA is believed to epimerize to an equilibrium mixture of approximately 9:1 (-)-JA:(+)-7-iso-JA. The actual equilibrium concentration *in planta* is unknown. Consequently, analysis of jasmonates isolated from plants should indicate which isomers are being analyzed. Commercially available synthetic MeJA used in many experiments is composed of a 9:1 ratio (±)-MeJA:(±)-7-iso-MeJA. The methyl esters may be converted to the free acids with either basic hydrolysis or incubation with commercially available esterases. Enantiomers of JA and 12-oxo-phytodienoic acid (OPDA) can be resolved as their methyl esters using cyclodextrin stationary phases in gas chromatography (Laudert et al., 1997).

To unequivocally confirm the role of compounds in plant growth and development, physico-chemical means of analysis must be performed. During sample preparation and purification for analysis, sample losses will occur for example, through spillage and during partitioning and chromatography. To obtain accurate and reliable measurements, losses must be accounted for through the use of suitable internal standards. Synthetic JA or jasmonate analogs containing deuterium or ^{13}C have been used to quantify endogenous jasmonates by GC-MS selected ion monitoring. Mueller and Brodschelm (1994) derivatized jasmonates to the pentafluorobenzyl esters for quantitation by GC-MS-NICI and reported a limit of detection of approx. 500 fg. The presence of the fluorine atoms accounts for the increased sensitivity of this method. The positive and negative ion electrospray mass spectra of several jasmonate amino acid conjugates were determined by combined HPLC-MS (Schmidt et al., 1995). Creelman et al., (1992) used (^{13}C,$^{2}H_3$)-MeJA while Gundlach et al., (1992) used 9,10 dihydrojasmonic acid to estimate JA levels. However, use of compounds, which are structurally similar to the compound being measured, may under- or over estimate endogenous levels unless the recovery efficiencies are identical. To circumvent this problem, Creelman and Mullet (1995) synthesized (2-^{13}C)-JA and used it to measure JA in soybean tissue whereas Baldwin et al., (1997) made (1,2-^{13}C)-JA with a molecular weight two mass units higher than endogenous JA. Stelmach et al., (1998) synthesized (2H5)-12-oxo-phytodienoic acid (OPDA) from 17(2H2), 18(2H3)-linolenic acid (LA) with lipoxygenase (LOX) and allene oxide synthase (AOS).

It is becoming apparent that oxylipins in addition to JA are important in plant growth and development. Weber et al., (1997) developed a generalized quantitative extraction procedure to isolate oxylipins. The procedure is simple and involves tissue homogenization, solid phase extraction and solvent partitioning. Extracts are methylated and analyzed by gas chromatography-mass spectrometry to give an oxylipin "profile" or signature.

OXYLIPINS IN *ARABIDOPSIS*

Although JA levels in unwounded *Arabidopsis* leaves are low, they increase upon wounding (Bell et al., 1995; McConn et al., 1997). However, when one begins to compare the oxylipin profile between *Arabidopsis* and other species and tissues, a complex picture begins to emerge. With other species JA levels are highest in young growing tissue and in flowers (Creelman and Mullet, 1995; Hause et al., 2000). Quantitation of oxylipins in unwounded and wounded leaves revealed complex differences between *Arabidopsis* and potato (Weber et al., 1997). For example, levels of OPDA were significantly greater than JA in unwounded *Arabidopsis* leaves yet in potato the levels of these two compounds were roughly equivalent. In wounded *Arabidopsis* leaves, JA levels were significantly higher than OPDA levels, yet in wounded potato leaves OPDA levels were higher than JA (Weber et al, 1997). In tomato, a similar complex situation exists between leaves and flowers (Hause et al., 2000). In tomato flower pistil, the level of OPDA is much higher than JA, yet in flower stalks JA levels are greater than OPDA (Hause et al., 2000). Thus, different species, tissues and stresses may show different oxylipin profiles. More work is needed to characterize how oxylipin profiles differ in different plant tissues, how development and stress may influence these profiles, and to understand how these changes modulate plant growth and development

Several compounds closely related to JA and its precursors are found in *Arabidopsis*. Using a method to profile the oxylipin content of *Arabidopsis*, Weber et al., (1997) characterized a 16-carbon molecule and identified it as dinor-oxo-phytodienoic acid (dnOPDA). Because dnOPDA was not detected in the hexadecatrienoic fatty acid (16:3) deficient *Arabidopsis* mutant *fad5*, Weber et al., (1997) concluded that dnOPDA was derived directly from plastid 16:3 rather than by b-oxidation of OPDA. Wounding caused an increase in dnOPDA in *Arabidopsis* leaves. Interestingly, exogenous dnOPDA increased the production of a-ketol formation in crude *Arabidopsis* leaf extracts from 16:3. This observation implies that dnOPDA may play

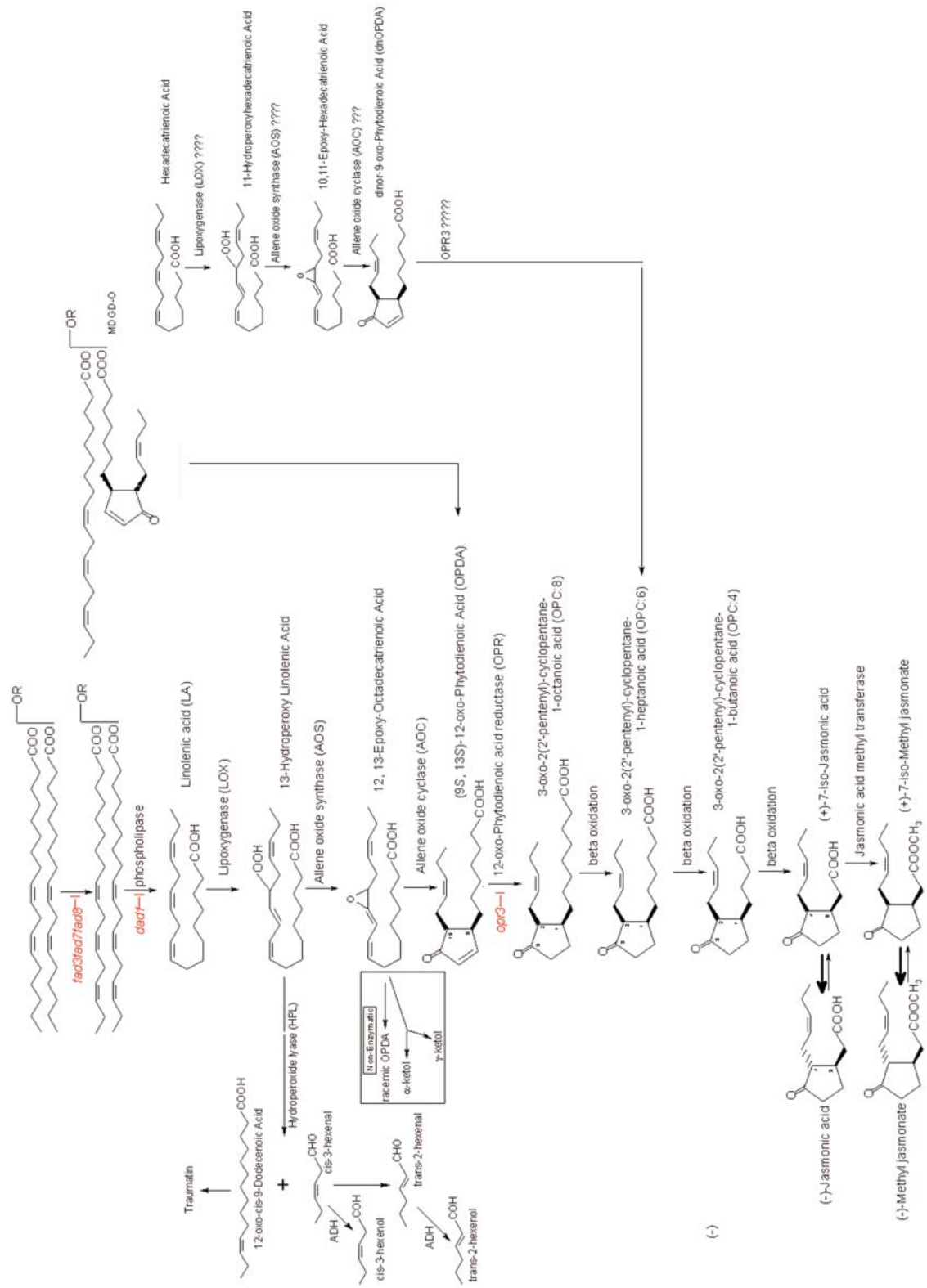

Figure 1. Biosynthetic pathway of oxylipins in *Arabidopsis*. It is postulated that signals (such as elicitors) interact with a membrane receptor causing the eventual production of 13-hydroperoxylinolenic acid. Production of 13-hydroperoxylinolenic acid is believed to occur with the release of linolenic acid via either a phospholipase or lipase followed by oxidation by lipoxygenase (LOX), but a preliminary oxidation of linolenic acid while still esterified to a phospholipid and subsequent release by a lipase cannot be ruled out. 13-hydroperoxylinolenic acid can then be catabolized by hydroperoxy lyase (HL) eventually forming volatile aldehydes and traumatic acid or via peroxygenase pathway to cutin monomers. Jasmonic acid arises from 13-hydroperoxylinolenic acid via an allene oxide synthase (AOS) and allene oxide cyclase (AOC) dependent pathway with 12-oxo-phytodienoic acid (OPDA) as an intermediate. Jasmonic acid then acts to modulate gene expression otr can be further catabolized.

a role in modulating flux through its biosynthetic pathway (Weber et al., 1997).

Gundlach and Zenk (1998) investigated whether oxylipins derived from linoleic acid (18:2) were significant in plant growth and development (Blechert et al., 1995). Early work had established that dihydrojasmonic acid (dh-JA) is found in low amounts in one plant species (*Vicia faba*) and 18:2 could be converted to dihydro-OPDA (dh-OPDA) by crude extracts (summarized in Gundlach and Zenk, 1998). In tissue culture, some plant species do respond to dh-JA by producing putative defense compounds (Gundlach and Zenk (1998). However, no dihydro oxylipins were detected in several other plant species. Furthermore, allene oxidase cyclase does not produce dh-OPDA from allene oxide derived from 18:2 (Gundlach and Zenk, 1998). Spontaneous cyclization of the 18:2 derived allene oxide may occur, or allene oxidase cyclase may have different substrate specificity. Whether this pathway is important to *Arabidopsis* remains to be seen.

While the majority of oxylipins occur in free form, Stelmach et al., (2001) demonstrated that crude lipid from *Arabidopsis* leaves would release OPDA when subjected to alkaline hydrolysis. This lipid fraction released OPDA enzymatically by *sn*1-specific, but not by *sn*2-specific, lipases. Mass spectrometric, and NMR spectroscopic data allowed Stelmach et al., (2001) to establish the structure as *sn*1-*O*-(12-oxophytodienoyl)-*sn*2-*O*-(hexadecatrienoyl)-monogalactosyl diglyceride (MGDG-O). Wounded leaves show a transient increase in the level MGDG-O. In *A. thaliana*, the major fraction of OPDA occurs esterified at the *sn1* position of the plastid-specific glycerolipid, monogalactosyl diglyceride.

A large fraction of MGDG has LA at the *sn*1 position. MGDG-O could be generated from the enzymes similar to LOX and AOS acting on MGDG. Stelmach et al., (2001) was unable to convert MGDG to MGDG-O *in vitro* using soybean LOX and *Arabidopsis* AOS. Many reasons could exist for the lack of conversion with this enzyme mixture, such as the *in vivo* enzymatic activities may differ from those used or that MDGD is required to be in a particular membrane environment in order to produce MDGD-O. Alternatively, free OPDA could be synthesized first and then incorporated into membrane lipid.

Several amino acid conjugates of JA also exist. In barley leaves, water stress increased the levels of JA conjugates of isoleucine, leucine, and valine (Kramell et al., 1995; Kramell et al., 2000). In *V. faba*, isoleucine and tryptophan conjugates have been found in leaves and flowers (Bruckner et al., 1988) . No reported examples of amino acid conjugates have been described for *Arabidopsis*.

The “green” odor associated with wounded plant tissue arises, in part, from volatile six carbon aldehydes. These aldehydes arise from the action of hydroperoxide lyase on either 18:3 or 18:2 hydroperoxides to give a 12 carbon acid (which gives rise to traumatin) and a 6 carbon aldhehyde. Either *cis*-3-hexenal or *n*-hexanal is produced, depending on whether 18:3 or 18:2 hydroperoxide is the substrate for hydroperoxide lyase. Other volatiles are present such as *trans*-2-hexenal (produced by the isomerization of *cis*-3-hexenal) as well as the corresponding 6 carbon alcohols hexanol and 3-hexenol (Bate et al., 1998).

BIOSYNTHETIC PATHWAYS FOR OXYLIPIN PRODUCTION AND REGULATION

An increased understanding of the temporal and spatial regulation of oxylipin biosynthesis pathways as well as an understanding of how oxylipins (in particular jasmonates) participate in signaling networks has occurred with the cloning of several biosynthetic genes from *Arabidopsis*. For example, the biosynthesis of jasmonates begins with the production of LA (Figure 1). This fatty acid is converted to 13-hydroperoxylinolenic acid by lipoxygenase. Dehydration of 13-hydroperoxylinolenic by allene oxide synthase forms an unstable epoxide, which is cyclized by a sterospecific allene oxide cyclase to form 12-oxo-phytodienoic acid (OPDA). Following reduction by OPDA reductase and three steps of beta-oxidation, 3(R), 7(S)-JA is formed. Jasmonic acid can be catabolized to form MeJA and numerous conjugates and catabolites, which may have biological activity (Hamberg and Gardner, 1992).

The Production of Linolenic acid

The *A. thaliana fad3-2 fad7-2 fad8* mutant has very low levels of linolenic acid and is unable to accumulate JA in response to wounding (McConn and Browse, 1996; McConn et al., 1997). Application of linolenic acid to plants results in accumulation of JA (Farmer and Ryan, 1992). This indicates that the level, distribution or availability of linolenic acid could determine the rate of JA biosynthesis.

Plant membranes, especially chloroplast membranes, are a rich source of linolenic acid esterified in glycerolipids and phospholipids. This has led to the suggestion that increases in JA in wounded leaves could result from the activation of phospholipases that release LA from membranes (Farmer and Ryan, 1992). In general, rapid, transient changes in membrane lipids occur when leaves are wounded. For example, levels of polar lipids decrease and levels of lysophospholipids and phospatidic acid (PA)

increase. Free fatty acids such as linolenic and linoleic acid also increase (Conconi et al., 1996; Lee et al., 1997; Ryu and Wang. 1998).

PA can be generated by the action of phospholipase D (PLD) or by PLC followed by diacylglycerol kinase. PLA catalyzes the formation of fatty acids and lysophospholipids from phospholipids (Wang, 1999). Phospholipases are constitutively present in plant tissues and when released from organelles by wounding, immediately degrade membranes. It has been suggested that PLA, PLC, and PLD activities increase in wounded leaves, and that a systemic induction of their activity occurs in nearby unwounded leaves (Conconi et al., 1996; Ryu and Wang, 1998; Lee et al., 1997; Narvaez-Vasquez et al., 1999).

In *Arabidopsis* PLD is encoded by a heterologous gene family (Qin et al., 1997; Wang, 1999; Qin and Wang, 2002). Twelve PLD cDNAs have been isolated (divided into 5 classes based on homology, PLDα, PLDβ, PLDγ, PLDδ and PLDζ; Qin and Wang, 2002). PLDβ, PLDγ and PLDγ2 gene expression was induced by wounding, whereas PLDα1 gene expression was relatively unaffected by wounding. However, wounding does rapidly activate PLDα1. This activation results from the translocation of cytosolic PLD to membranes upon an increase in cytosolic Ca^{+2} caused by wounding (Wang et al., 2000).

In wounded leaves of *Arabidopsis* plants in which PLDα1 was suppressed by introducing a PLDα1 antisense gene, the levels of JA and the expression of two JA responsive genes (AtLox2 and AtVSP) were reduced. Interestingly, the expression of allene oxide synthase (AOS) and hydroperoxide lyase (HPL) genes in which PLDα was suppressed was similar to that seen in wounded wild-type plants (Wang et al., 2000). Hence, PLDα1 may modulate JA levels by either its action on phospholipases such as PLA or by modifying the expression of LOX2. However, other signaling mechanisms must exist because neither AOS nor HPL gene expression was altered in PLDα1 cosuppressed plants.

An acyl hydrolase, SAG101, has been suggested to play a role in leaf senescence (He and Gan, 2002). Overexpression of SAG101 caused accelerated senescence in Arabidopsis leaves while RNAi experiements delayed the appearance of senescence for 4 days. An acyl hydrolase has been proposed to be responsible for the release of –linolenic acid from phosphatidic acid in the wound-induced accumulation of jasmonic acid (Wang et al., 2000). However, the role of SAG101 in JA biosynthesis is not known since no measurements of JA were performed using plants overexpressing SAG101.

A *PLA1* gene (*defective in anther dehiscence1*; *DAD1*) has been identified in *Arabidopsis* which appears to have some of the characteristics required for an enzyme involved in the production of free LA for JA biosynthesis (Ishiguro et al., 2001). DAD1 contains a putative N-terminal plastid transit peptide and contains conserved motifs found in lipase active sites. However, expression of *DAD1* occurs primarily in stamen filaments. While wound induced expression of *DAD1* does occur, it should be noted that substantial JA accumulated in wounded leaves of *dad1* plants. Hence, DAD1 does not appear to play a role in defense responses to wounding and may function solely to provide JA needed for proper anther and pollen development. However, a strong increase in soluble PLA2 activity occurred in tobacco leaves infected with tobacco mosaic virus. This increase in activity preceded increases in the accumulation of OPDA and JA (Dhondt et al., 2000). In tomato, PLA2 activity was induced by wounding, systemin, and cell wall elicitors (Narvaez-Vasquez et al., 1999).

SAG101, a leaf senescence-associated gene, was cloned from an Arabidopsis leaf senescence enhancer trap line and functionally characterized. Reporter gene and RNA gel blot analyses revealed that SAG101 was not expressed until the onset of senescence in leaves. A recombinant SAG101 fusion protein overexpressed in Escherichia coli displayed acyl hydrolase activity. Antisense RNA interference in transgenic plants delayed the onset of leaf senescence for approximately 4 days. Chemically induced overexpression of SAG101 caused precocious senescence in both attached and detached leaves of transgenic Arabidopsis plants. These data suggest that SAG101 plays a significant role in leaf senescence.leaf senescence associated gene, SAG101,

Lipoxygenase

LOX catalyzes the conversion of linolenic acid to 13-hydroperoxylinolenic acid. Two different LOX genes, *AtLox1* (Melan et al., 1993) and *AtLox2* (Bell and Mullet, 1993), have been identified in *A. thaliana*. *AtLox1* is expressed in leaves, roots, inflorescence, and young seedlings, with the highest expression found in roots and young seedlings. Because *AtLox1* lacks obvious targeting sequences, this enzyme is most likely localized in the cytoplasm. In contrast, *AtLox2* is localized in chloroplasts (Bell et al., 1995). *AtLox2* mRNA levels are high in leaves and inflorescence but low in seeds, roots, and stems. The physiological role of this chloroplast lipoxygenase was analyzed by reducing LOX2 accumulation in transgenic plants (Bell et al., 1995). The reduction of *AtLox2* expression caused no obvious changes in plant growth. However, the wound-induced accumulation of JA observed in control plants was absent in leaves of transgenic plants lacking *LOX2*. Therefore, plastid localized *LOX2* is required for

wound-induced synthesis of jasmonates in *Arabidopsis* leaves.

Allene oxide synthase

The fate of 13-hydroperoxylinolenate produced by LOX is another key branch point in the jasmonate biosynthetic pathway (Figure 1). Hydroperoxide lyase (see below) will cleave 13-hydroperoxylinolenate to form volatile six carbon aldehydes and 12-oxo-dodecenoic acid (Vick, 1993). 13-hydroperoxylinolenate can also be used by peroxygenase to produce precursors of cutin molecules (Blée and Schuber, 1995). In contrast, production of jasmonates requires that 13-hydroperoxylinolenate be metabolized to the unstable intermediate allene oxide by allene oxide synthase (AOS). The *Arabidopsis AOS* has been cloned and characterized (Laudert et al., 1996; Laudert and Weiler, 1998; Kubigsteltig et al, 1999). AOS is a cytochrome P450 enzyme of the CYP74A subfamily (Song et al., 1993) and contains a putative N-terminal plastid targeting sequence (Laudert et al., 1996) and AOS synthase activity has been localized to the plastid outer envelope in spinach (Bleé and Joyard, 1996). *AOS* exists as a single copy within the *Arabidopsis* genome (Laudert and Weiler, 1998) and is expressed primarily in leaves and flowers. Wounding causes *AOS* expression to increase, as well as increasing AOS activity (Laudert et al., 1996; Laudert and Weiler, 1998) in both the directly damaged leaves and in the systemic tissue located distal to the treated leaves. *AOS* expression also appears to be induced by JA and OPDA (Laudert and Weiler, 1998; Kubigsteltig et al, 1999).

Over expression of *AOS* in transgenic *Arabidopsis* or tobacco plants did not alter JA levels in control unwounded leaves. Wounding, however, caused the production of significantly more JA in these plants (Laudert et al., 2000). This result suggests that in these two plants the biosynthetic pathway for JA is limited by the production of LA or 13-hydroperoxylinolenic acid. Interestingly, over expression of flax AOS in transgenic potato plants increased JA levels (Harms et al., 1995) indicating that the amount of AOS protein limits JA biosynthesis yet had no effect on the expression of JA responsive genes.

Hydroperoxide Lyase

Hydroperoxide lyase (HPL) cleaves lipid hydroperoxides formed from linoleic and linolenic acids to produce a C6 aldehyde and C12 oxo-acid. The Arabidopsis *HPL* was isolated using the bell pepper *HPL* sequence blasted against the *Arabidopsis* genome and EST databases (Bate et al., 1998; Rojo et al., 1998; Matsui et al., 1999). HPL is a cytochrome P450 with a strong similarity with the AOS amino acid sequence, however it resides in the CYP74B subfamily. The deduced amino acid sequence also contains a putative targeting sequence suggesting that HPL is targeted to the chloroplast envelope, consistent with the localization by enzymatic analysis (Blee and Joyard, 1996), *HPL* was expressed at relatively high levels in mature inflorescence, flower buds, flowers, and siliques, at lower levels in root tissue and cauline leaves and at substantially lower levels in rosette leaves (Bate et al., 1998; Matsui et al., 1999). Both wounding and MeJA treatment appear to cause induction of *HPL* expression in rosette leaves (Bate et al., 1998; Rojo et al., 1998; Matsui et al., 1999). Significant HPL activity was evident when 13-hydroperoxylinolenate was the substrate, whereas activity with 13-hydroperoxylinoleate was approximately 10-fold lower (Bate et al., 1998; Matsui et al., 1999). This indicates that another isozyme of HPL must exist which catalyzes the formation of n-hexanal from 13-hydroperoxylinoleate. Conversion between 6 carbon aldehydes to the corresponding alcohol proceeds through the action of alcohol dehydrogenase (ADH). Using an *adh* mutant of *Arabidopsis*, Bate et al., (1998) demonstrated differences in the levels of 6 carbon aldehydes and alcohols in the absence of ADH activity. Hexanol and 3-hexenol levels were reduced about 50% but the level of hexanol was about 10-fold higher. The altered profile of LOX-derived volatiles in the *adh* mutant did not have an effect on the steady-state levels of mRNA for AOS or LOX, whereas HPL enzymatic activity and *HPL* mRNA quantity were higher in the mutant relative to wild type. Hence, the products of the HPL catalyzed reaction may affect *HPL* regulation.

Allene Oxide Cyclase

Allene oxide cyclase (AOC) catalyzes the stereospecific cyclization of the unstable allene oxide to OPDA. OPDA

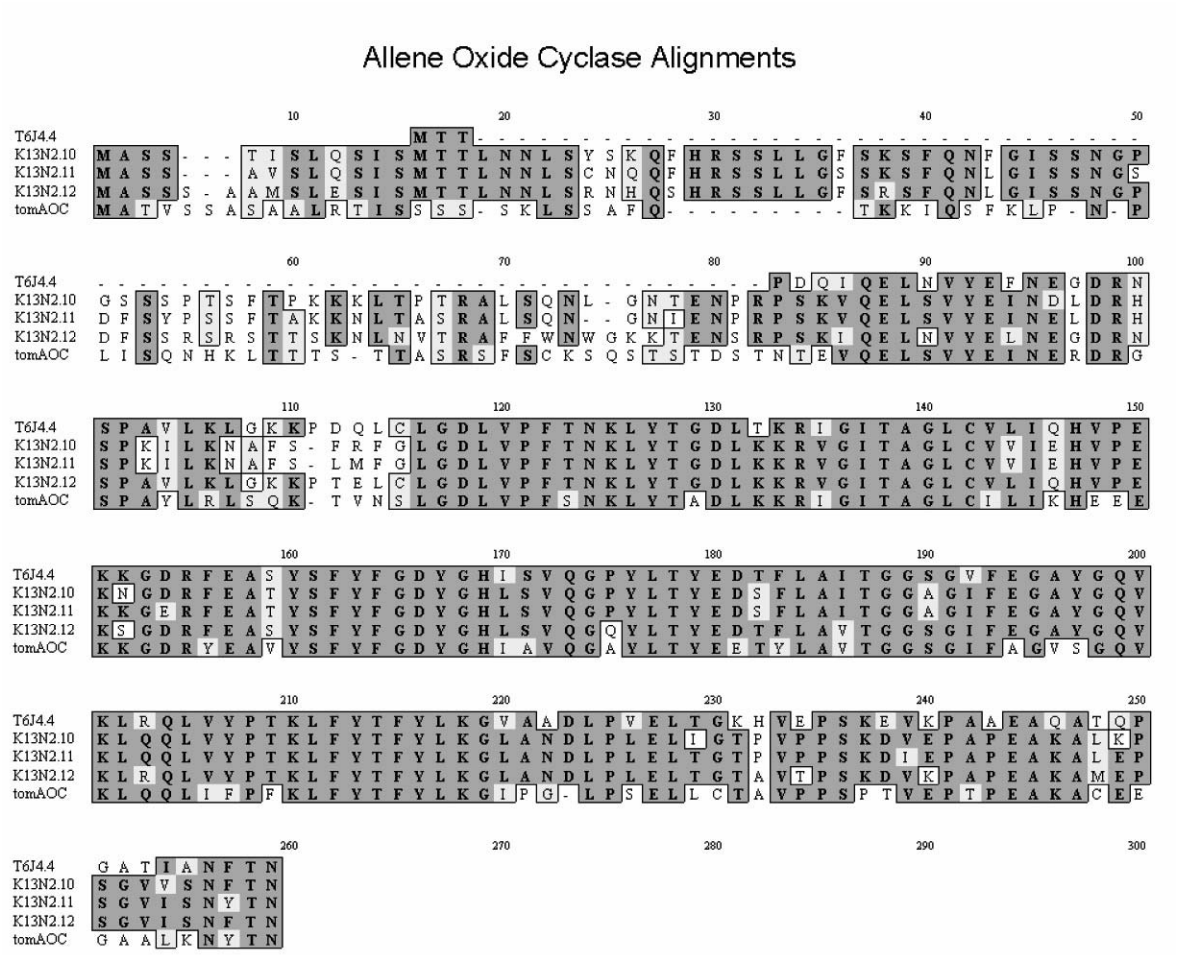

Figure 2. Alignment of putative *Arabidopsis* allene oxide cyclases with tomato allene oxide cyclase. Three *Arabidopsis* genes (T6J4.4, K13N2.11, K13N2.10) were aligned with tomato (tomAOC, Ziegler et al., 2000). Note that T6J4.4 appears to lack a plastid transit peptide. Dark grey indicates identical amino acids, light grey indicates similar amino acids. Alignments were performed using MacVector.

formed by this enzyme is the cis-(+) enantiomer having a 9*S*, 13*S* configuration. To date nothing has been published on AOC from *Arabidopsis*. However, several studies have been performed using purified AOC from *Zea mays* (Ziegler et al., 1997). Based on gel filtration, the enzyme appears to be a homodimer and is N-terminally processed (Ziegler et al., 1997; Ziegler et al., 2000). The amino acid sequence from corn was used to design degenerate primers used in the eventual isolation of a full length cDNA clone from tomato (Ziegler et al., 2000). Analysis of OPDA formed by both the corn and tomato AOC indicated that the correct enantiomer was formed (9S, 13S). The tomato AOC contains a plastid transit sequence and was localized within the chloroplast using immunohistochemistry (Ziegler et al., 2000). In tomato, *tomAOC* expression is highest in roots, flower buds, flower stalks (Hause et al., 2000). Expression was lower in stems, young leaves, and flower pistils (Hause et al., 2000). While no published information exists on AOC from *Arabidopsis*, using the tomato AOC amino acid sequence in a TBLASTN query of the published *Arabidopsis* genome reveals at least four putative AOC genes in the *Arabidopsis* genome (Figure 2). All four putative genes share an extremely high degree of homology with *tomAOC*. Three genes (K13N2.10, K13N2.11, and K13N2.12) also contain structural features similar to those found in plastid signal peptides: (a) the first amino acid after the start methionine is alanine, (b) no charged amino acids in the first 10 residues, and (c) a large number of ser-

ines in the first 50 amino acids (28% for K13N2.10 and 30% for K13N2.11 and 26% for K13N2.12; von Heijne et al., 1989). Interestingly, K13N2.10, K13N2.11, and K13N2.12 are located next to each other on chromosome 3. No such plastid signal peptide was found in T6J4.4 (GenBank Accession AC011810). This suggests that either T6J4.4 is either not targeted to plastids or that the gene prediction program is in error.

12-oxo-Phytodienoate Reductase

12-oxo-phytodienoate reductase catalyzes the reduction of OPDA to 3-oxo-2(2'-pentenyl)-cyclopentane-1-octanoic acid (OPC:8). Three isozymes of OPR have been identified in *Arabidopsis* (Schaller and Weiler, 1997b; Biesgen and Weiler, 1999; Sanders et al., 2000; Schaller et al, 2000; Stintzi and Browse, 2000). *OPR1* and *OPR2* were identified using sequence information from *OPR1* from *Corydalis sempervirens* (Biesgen and Weiler, 1999, Schaller and Weiler 1997a, Schaller et al, 2000). *OPR3* was identified as a cDNA up-regulated by brassinolide (Mussig et al., 2000) and during the characterization of T-DNA tagged fertility mutants (*opr3*, Stintzi and Browse, 2000; *dde1* (*delayed dehiscence1*), Sanders et al., 2000). Two genes, *OPR1* and *OPR2* encode proteins with little ability to reduce the natural (9*S*, 13*S*) isomer of OPDA to OPC:8 whereas OPR3 efficiently catalyzes this step (Schaller et al, 2000). Hence, OPR3 encodes the protein involved in JA biosynthesis.

OPR1 and *OPR2* are predominantly expressed in roots (Biesgen and Weiler, 1999). In contrast, *OPR3* is expressed in all plant parts including flowers and leaves (Sanders et al., 2000; Stintzi and Browse, 2000). *OPR3* expression is also induced by brassinosteroids, JA, and by a variety of stimuli like UV irradiation, touch, and wounding (Mussig et al., 2000). All OPRs from *Arabidopsis* and *C. sempervirens* share significant sequence similarity with Old Yellow Enzyme, a flavin-containing enzyme reducing double bonds of α,β-unsaturated carbonyls in a NADPH dependent fashion (Schaller and Weiler, 1997b). OPR3 contains an SRL peptide sequence at its carboxyl terminus suggesting that it is localized in the peroxisome (Hayashi et al., 1996). No targeting sequences have been determined for OPR1 and OPR2.

Beta-Oxidation

The β–oxidation pathway contains enzymes that degrade fatty acids by the sequential removal of two carbon units. β–oxidation in plants appears to take place almost entirely in glyoxysomes and peroxisomes. Research on β–oxidation has focused primarily on its role during mobilization of storage compounds from tissues such as castor bean endosperm and the enzymes the enzymes of the plant β–oxidation have been well characterized biochemically (Gerhardt 1992). Three 3-keto-acyl thiolases have been identified in Arabidopsis (Rocha et al., 1996; Hayashi et al., 1998). Two proteins, *PKT1* and *PKT2*, arise via alternative splicing. Another thiolase, *ped1*, was isolated when mutaganized Arabidopsis seedlings were screened for growth in the presence of toxic levels of 2,4-dichlorophenoxybutyric acid (2,4-DB). 2,4-DB is metabolized to produce the herbicide 2,4-D by the action of peroxisomal fatty acid ß-oxidation.

Unpublished data by Afithile and Hildebrand (cited in He et al., 2002) indicates that *PED1* is needed for JA biosynthesis in wounded leaves. The *abnormal inflorescence meristem1* (*aim1*) mutation alters affects inflorescence and floral development in *Arabidopsis*. During the transition to flowering, abnormal flower meristems are produced which result in *aim1* plants with reduced fertility (Richmond and Bleecker, 1999). AIM1 appears to posses enoyl-CoA hydratase activity, another enzyme in the β–oxidation pathway (Richmond and Bleecker, 1999). However, its role in JA biosynthesis remains unclear because JA levels have not been determined in *aim1* mutant plants.

Jasmonic Acid Carboxyl Methyltransferase

Seo et al. (2001) cloned an S-adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase (JMT) from *A. thaliana*. Comparison of the *JMT* cDNA sequence with the corresponding genomic sequence indicates that JMT is a putative 45-kDa polypeptide consisting of 389-aa residues with 4 exons and contains motifs conserved among O-methyltransferases. No transit signal peptides are apparent in the sequence so JMT is presumably a cytoplasmic enzyme. *JMT* mRNA was expressed in most parts of mature plants, particularly in rosettes, cauline leaves, and opening flowers. *JMT* mRNA was not detected in young seedlings. Both wounding and MeJA also induced JMT expression.

Transgenic *Arabidopsis* plants overexpressing *JMT* contained elevated levels of MeJA yet their JA content was unaltered. In these transgenic plants, expression of various jasmonate-responsive genes was elevated in the absence of wounding or jasmonate treatment. Transgenic plants also showed enhanced level of resistance against the virulent fungus *Botrytis cinerea*. In contrast, overexpression of a flax *AOS* cDNA in transgenic potato plants did not cause overexpression of wound- or jasmonate-responsive genes, even though the plants contained higher levels of JA (Harms, 1995). In addition**,** levels of JA in transgenic *Arabidopsis* plants overexpressing *AOS* were not increased unless the tissues were damaged (Laudert et al., 2000). These data suggest that, while *AOS* may be a central branch point in the pathway to JA, the expression of *JMT* (and subsequent production of MeJA) is important for the induction of genes such as *AtLox2* and *AOS*. This positive feedback mechanism could provide more substrate for MeJA synthesis and further induce jasmonate-responsive genes.

OXYLIPIN FUNCTION AND SIGNAL TRANSDUCTION IN *ARABIDOPSIS*

Over past decade, JA has emerged as a new class of plant growth regulator. JA and its derivatives have physiological effects at very low concentrations and indirect evidence suggests that it be transported throughout the plant. To date, jasmonates have been shown to cause both inhibitory and promotive effects on plant morphology and physiology, some of which are similar to the effects induced by other well known phytohormones such as abscisic acid and ethylene. Exogenous applications of JA have shown to inhibit embryogenesis, seed germination, seedling growth, root growth, photosynthetic pigments and photosynthetic activities, pollen germination and flower bud formation. In addition to its inhibitory effects, jasmonates have also been shown to promote cell differentiation, breaking seed dormancy, adventitious root formation, stomatal closure, respiration, pollen germination, fruit ripening, pericarp and foliar senescence, foliar abscission, tuber formation, tendril coiling, microtubule disruption, ethylene biosynthesis, and protein synthesis (Sembdner and Parthier, 1993; Creelman and Mullet, 1997; Reymond and Farmer, 1998). In addition to the above mentioned developmental and physiological roles, JA and its methyl esters have also been shown to protect plants against mechanical or herbivorous insect-driven wounding (McConn et al., 1997), pathogens (Dong, 1998; Penninckx et al., 1998; Thomma et al., 1998; Vijayan et al., 1998), osmotic stress (Kramell et al., 2000) and ozone (Rao et al., 2000b).

Mode of Action

The JA signal transduction pathway has just begun to be understood. It is presumed that JA (and other oxylipins) interact with receptors and activate pathways causing changes in transcription, translation and other responses mediated by JA. JA receptors and other components of the signal transduction pathway are most likely to be discovered through analysis of mutants that are insensitive or altered in their response to JA. To date, up to five different classes of JA insensitive mutants have been identified; *coi1*, *jar1*, *jin1*, *jin4, cev1* and *cex1*; Benedetti et al., 1995; Berger et al., 1996; Ellis and Turner, 2001; Staswick et al., 1992; Xu et al., 2001). Genetic studies were unable to determine if *jin4* and *jar1* were allelic (Berger et al., 1996). The mutants *jar1*, *jin1* and *jin4* were recovered using a root growth screen (wild type *A. thaliana* root growth is inhibited by 1-10 mM JA). In contrast, *coi1* was identified because plants were resistant to coronatine (Feys et al., 1994). Coronatine is a chlorosis-inducing toxin, which has a chemical structure and biological activity similar to JA. The *coi1*mutant also shows a MeJA insensitive root growth phenotype.

While these mutants cause loss of function and presumably define genes that function as positive regulators, two groups developed screens to identify negative regulators (*cev1*, Ellis and Turner, 2001; *cex1*, Xu et al., 2001). Ellis and Turner (2001) screened mutagenized seeds containing the promoter of a JA responsive gene fused to a reporter gene, while Xu et al. (2001) screened mutagenized seeds for mutants that constitutively exhibited phenotypes of plants treated with JA (such as JA specific gene expression and stunted hypocotyl or root growth). Information regarding whether *cex1* is an allele of *cev1* is not definitive. Both *coi1*/*coi1*; *cev1*/*cev1* and *coi1*/*coi1*; *cex1*/*cex1* plants had short roots. Anthocyanin production was suppressed in *coi1*/*coi1*; *cev1*/*cev1* seedlings (Ellis and Turner, 2001) but no information on the anthocyanin content of *coi1*/*coi1*; *cex1*/*cex1* seedlings were reported (Xu et al., 2001). Both mutants do constitutively exhibit JA responsive phenotypes such as enhanced *AtVSP*, *Thi2.1*, and *PDF1.2* expression, stunted roots, and in the case of *cev1*, enhanced resistance to powdery mildew.

In a similar manner, Hilpert et al., (2001) screened mutagenized seeds containing the promoter of the JA respon-

sive gene *Thi2.1* fused to *bar* gene. The *bar* gene codes for an enzyme which enables plants to grow on the herbicide Basta. Nine *cet* (*constitutive expression of thionin*) mutants were isolated. Four mutants were dominant, four were recessive, and one did not survive past the primary leaf stage. Most homozygous mutants grew poorly with necrotized leaves and several died before flowering. Of the mutants which survived, two mutants (*cet1* and *cet3*) had JA and OPDA levels were significantly higher than those found in wild-type plants. In the case of *cet1* levels were almost 40 times higher than wild-type plants. In *cet4-1* levels of JA and OPDA were similar to wild-type plants. In *cet4-1* plants, the upregulation of *Thi2.1* could result from enhanced sensitivity to JA or to activation of a component of the JA signalling pathway downstream of JA.

Jensen et al. (2002) also used a promoter::reporter gene fusions to isolate jasmonate signalling mutants. They screened a population of gamma irradiated AtLOX2 promoter::luciferase progeny to identify three recessive mutants that underexpressed the reporter gene (*jue1*, *jue2* and *jue3*; *jasmonate underexpressing*) as well as two recessive mutants (*joe1* and *joe2*; *jasmonate overexpressing*) that overexpress the reporter gene. A mapping experiment places *joe2* in the same region as *cev1* (Ellis and Turner, 2001). While they might be alleles, they both have different phenotypes. Crossing experiments of *joe1* and *joe2* with *coi1*and analysis of progeny indicate that *JOE1* and *JOE2* appear to act upstream of *COI1*.

Of the mutants identified in the JA signaling pathway, only the gene corresponding to *COI1* has been isolated (Xie et al., 1998). This gene is of interest because it indicates that a specific proteolysis step is required for the perception of JA. Sequence analysis of the deduced amino acid sequence of the COI1 protein indicates the presence of an F-box motif and several leucine rich repeats (Xie et al., 1998). Leucine rich repeats are short sequence motifs believed to be involved in protein-protein interactions (Kobe and Deisenhofer, 1994). The F-box is a Skp1-interacting domain (Bai et al., 1996) first identified in Cdc4 and cyclin F. This domain has been identified in several other regulatory proteins including Skp2 (Bai et al., 1996), Grr1 (Li and Johnston, 1997), and TIR1 (Ruegger et al., 1998). Skp1 and F-box containing proteins may function by connecting regulatory proteins with ubiquitin-mediated proteolysis (Skowyra et al, 1997) by serving as components of ubiquitin-protein ligase to facilitate transfer of ubiquitin from E2 to a targeted regulatory protein (Figure 3A). COI1 may act by promoting the degradation of a protein that exerts a negative regulatory effect in JA perception. With this identification of a component of the ubiquitin dependent protein degradation pathway in plants, studies utilizing yeast two hybrid systems should be able identify other components such as *SKP1* homologs.

Other studies utilizing a pharmacological approach have been useful in identifying components of the JA signaling pathway. Genes regulated by JA require *de novo* protein synthesis (Rojo et al., 1998). Rojo et al. (1998) used fairly specific kinase and phosphatase inhibitors to determine the role of protein phosphorylation in JA signaling. Rojo et al. (1998) were able to identify a staurosporine sensitive protein kinase which negatively regulates the pathway and a protein phosphatase 2A sensitive to okadaic acid which activated JA responsive gene expression (Figure 3B). Using a AtLox2 promoter::luciferase gene fusion, Jensen et al. (2002) demonstrated that reporter gene activity was also induced by staurosporine and antagonized by okadaic acid. Interestingly, reporter gene induction and endogenous LOX2 expression by staurosporine was present in *joe1* yet absent in *joe2* (Jensen et al., 2002). These authors suggest that *joe1* acts prior to the phosphorylation step and that *joe2* may cause the inactivation of a kinase or its substrate. Rojo et al. (1998) has suggested that *jin1*, *jin4* and *coi1* affect steps prior to this phosphorylation step. They also identified a JA independent pathway, which turned on a different set of wound responsive genes. Here, in contrast, a protein kinase positively regulated the JA independent pathway while the protein phosphatase negatively regulated it (Rojo et al., 1998). In another pharmacological study, Leon et al., (1998) demonstrated that the JA-dependent and JA-independent pathways were regulated differently by calcium and calmodulin. Mobilization of intracellular calcium pools blocked induction of JA responsive genes by wounding or JA but not gene expression in the JA-independent pathway. Antagonists of calmodulin also blocked JA responsive gene induction and increased gene expression in the JA-independent pathway (Leon et al., 1998). The action of calcium and calmodulin appeared to be downstream of the reversible phosphorylation events in both JA-dependent and JA-independent pathways (Rojo et al., 1998).

Natural Variation

Identification of mutant plants resistant to various hormones is a powerful approach for studying plant hormone signaling. Most studies, to date, have utilized forward and reverse genetic approaches in a few wild type accessions such as Col-0, L*er*, Ws, etc. Since the detection of a mutant phenotype is dependent on the wild-type background, it is likely that a null or a weak allele may not be detected and some phenotypes may appear only in certain backgrounds. As an alternative to generating mutants by using classical approaches, Rao and Davis (1999) and Rao et al., (2000b) have identified a JA insensitive ecotype

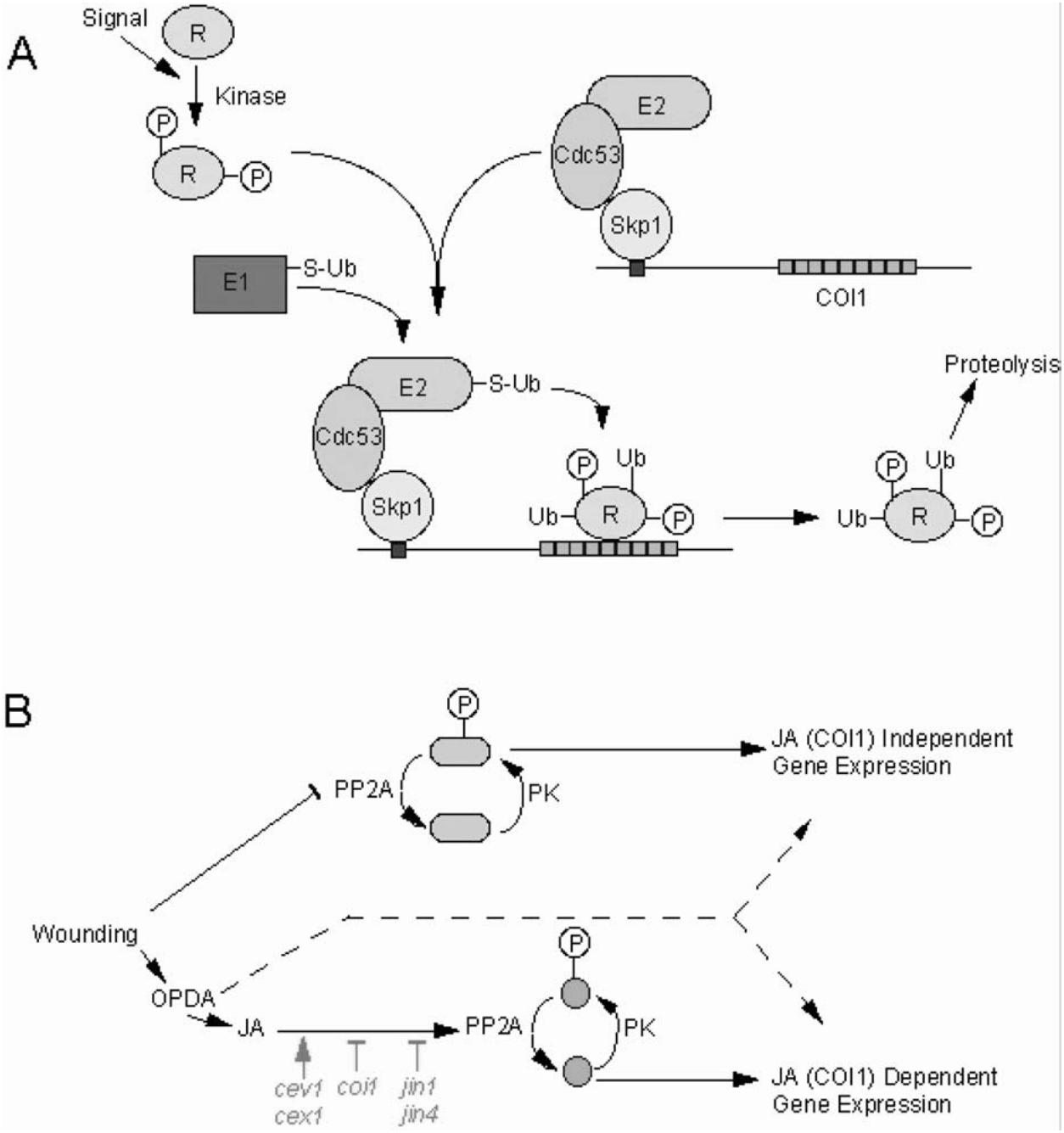

Figure 3. Oxylipin Signaling pathway in *Arabidopsis*.
Hypothetical model showing the function of COI1 in ubiquitin (UB) mediated proteolysis (A). A developmental or environmental signal causes the phosphorylation of R, a regulatory protein. Phosphorylated R binds to COI1 via a leucine repeat region (blue squares). A SCF complex (Skp1, Cdc53, and COI1) act as a ubiquitin ligase to transfer ubiquitin to R. After polyubiquination, R is released and destroyed by the proteosome (modified from Creelman, 1999). A hypothetical model for wound signaling (B) in *Arabidopsis* showing JA-dependent (lower) and JA-independent (upper) pathways (modified from Rojo et al., 1998 and others). The *cev1* and *cex1* mutants positive activate the JA dependent pathway. Other mutants, such as *coi1*, *jin1*, and *jin4*, prevent JA induced gene expression.

based on its hypersensitivity to an atmospheric pollutant, ozone (O_3). Subsequent screening of 125 different ecotypes have identified several ecotypes that are insensitive to MeJA (Rao and Davis, unpublished results). In a separate study aimed at monitoring the natural variation in glucosinolate contents, Kliebenstein et al., (2002) have identified several ecotypes that have defects in MeJA signal transduction pathways. Isolation of the loci that contributes to the differential response of JA signaling pathways may increase our understanding on the role of JA in plant growth development and stress tolerance.

Defense Responses

The formation of hydroperoxy derivatives of polyunsaturated fatty acids (PUFAs) represents the first step in the synthesis of oxidized PUFAs, the oxylipins. Oxylipins such as JA and its methyl esters occur ubiquitously in all plant tissues and constitute a major signaling compounds in influencing plant growth and development, and plant stress responses (Creelman and Mullet 1997). First, several studies have demonstrated increased oxylipin levels in *Arabidopsis* leaves in response to wounding or attack by pests or pathogens (McConn et al, 1997; Reymond et al., 2000; Schaller, 2001; Stintzi et al., 2001), ozone (Rao et al., 2000b), UV (Conconi et al., 1996) and osmotic stress (Kramell et al., 2000). Second, JA activates the expression of several genes involved in defense reactions (Reymond et al., 2000; Schenk et al., 2000; Sasaki et al, 2001). Finally, mutants defective in JA biosynthesis or perception also show altered responses when challenged by insect pests or fungal pathogens (Ellis and Turner, 2001; McConn et al, 1997; Pieterse et al., 1998; Staswick et al., 1998; Vijayan et al, 1998) and other abiotic stress factors (Rao et al., 2000a). Surprisingly, the *opr3* mutant shows wild-type resistance to insect and fungal pests (Stintzi et al., 2001). This strongly implies that OPDA is a signaling molecule in addition to JA. Furthermore, microarray analysis of genes induced in wounded leaves from *opr3* plants indicated that OPDA was sufficient to alter expression of several JA induced genes and that OPDA could activate JA/COI1 independent gene expression. To explain this observation, Stintzi et al. (2001) argue that the reactive electrophilic nature of OPDA and dnOPDA may be a factor in mediating gene expression (Vollenweider et al, 2000).

Crosstalk

Although JA is a major phytohormone believed to regulate many plant physiological processes, recent studies increasingly suggest that JA-mediated responses are strongly influenced by the type and the nature of interaction with other signaling pathways such as ethylene and salicylic acid (SA; Dong, 1998; Reymond and Farmer, 1998; Genoud and Metraux, 1999). For example, there is abundant evidence in the literature suggesting that SA and JA, depending on the plant species and stimuli, antagonize each other or in some instances may act synergistically (Xu et al., 1994; Niki et al., 1998). The antagonistic relation between the JA and SA signaling pathways is well documented in the studies of wound responses in plants. SA has been shown to inhibit both JA biosynthesis (Pena-Cortes et al., 1993) and JA-dependent induction of proteinase inhibitor genes (Doares et al., 1995), while both JA and ethylene are required to negate the negative influence of SA on JA-dependent signaling pathways (O'Donnell et al., 1996). Analogous to the interdependence of SA and JA, recent studies have revealed that ethylene function synergistically with both SA and JA. The JA-induction of *pin* gene expression was shown to require ethylene (O'Donnell et al., 1996), and a defensin gene was shown to be induced in a SA-independent yet ethylene- and JA-dependent manner (Penninckx et al., 1996; Penninckx et al., 1998).

Crosstalk Revealed by Gene Expression Studies

Wounding or the application of JA to *Arabidopsis* causes large changes in mRNA populations (Reymond et al., 2000; Schenk et al., 2000; Sasaki et al, 2001; Stintzi et al., 2001). Reymond et al. (2000) used 150 and Schenk et al. (2000) 2,375 PCR amplified EST's arrayed onto glass slides. Both groups selected EST's believed to be involved in defense or regulatory pathways. Sasaki et al. (2001) used 2,880 EST's generated from *Arabidopsis* shoots gridded onto nylon. Both wounding and exogenous JA cause the coordinated expression of some gene families, for example amino acid metabolism, secondary metabolism, wound related genes, and JA biosynthesis (Reymond et al., 2000; Sasaki et al, 2001; Schenk et al.,

2000). Using *coi1* seedlings, Reymond et al., (2000) demonstrated that the existence of a JA-independent pathway for the induction of wound gene expression similar to results found in pharmacological studies (Rojo et al., 1998). Interestingly, ethylene was not needed for the induction of this *COI1*-insensitive wound gene expression. However, several genes induced by mechanical wounding were also induced by dehydration stress. Note that Schenk et al., (2000) observed a large number of gene that were induced or repressed by either salicylic acid (SA) or ethylene were also induced or repressed by JA. Out of a total of 2375 EST's, 55 genes were found induced by MeJA and SA treatments, while 28 genes were repressed by both treatments. Although JA is widely believed to act synergistically with ethylene, only 25 genes were co-induced by MeJA and ethylene treatments (Schenk et al., 2000). The amount of crosstalk between JA and SA on gene expression was somewhat surprising considering the existence of separate JA and SA defense pathways (Thomma et al., 1998; Wees et al., 2000) and that JA and SA are considered be antagonists. However, it should also be noted that Schenk et al., (2000) have also found 8 genes that are induced by SA but repressed by MeJA suggesting that the antagonism between SA and MeJA may be specific to particular genes.

To obtain information on the regulatory network of JA-responsive genes, Sasaki et al., (2001) have screened 2800 cDNA clones and identified several MeJA responsive genes that are known to be involved in stress responses, JA biosynthesis, phytohormone signaling, disease and wound response and senescence. Interestingly this study has also shed light on the nature of crosstalk of JA with other signaling compounds. MeJA treatments have induced a gene encoding dioxygenase-like protein, which is homologous to E8 protein in tomato. Since E8 protein has been shown to regulate ethylene biosynthesis during fruit ripening (Kneissl and Deikman, 1996), it has been postulated that an intricate mechanism of interaction exists between JA and ethylene signal transduction. In addition, these studies have also demonstrated that auxins have an antagonistic effect to jasmonates (Rojo et al., 1998). MeJA treatments have induced the transcript levels of IAR3, which encodes IAA-Ala hydrolase with in 1 h or treatment suggesting that MeJA initially up-regulates the production of active IAA which then subsequently represses the transcription of other JA-responsive genes (Sasaki et al., 2001).

It is estimated that Arabidopsis genome contains at least 1533 transcription factors (~5.9% of its genome; Riechmann et al., 2000). Since many of the complex plant developmental and stress responses are regulated by several transcription factors, Chen et al., (2002) have performed microarray experiments with chips containing 402 known and putative transcription factors. These studies have identified both negative and positive interactions between JA/ethylene and SA signaling pathways in response to pathogen infection. Chen et al., (2002) have identified a group of transcription factors (for example, *GBF3*) that are repressed in SA signaling mutants, while they were induced in JA/ethylene signaling mutants. Further, they have also identified a group of transcription factors (for example, *RAP2.6* and *ERF*) that were reduced by mutations in JA/ethylene signaling pathways but induced in SA signaling mutants. In addition to the negative interactions, a group of transcription factors such as *AtERF1* and *AtERF2* were reduced by pathogen infection in all JA/ethylene and SA signaling mutants. The same study have also identified several transcription factors that are not responsive to JA/ethylene and SA (for example, R2R3-MYB transcription factor) suggesting that plant defense signaling pathways are complex and that JA possibly interacts with other signaling pathways.

Crosstalk Revealed by Signaling Mutants

Many of the documented JA-mediated responses were identified by application of jasmonate to plants, sometimes at non-physiological levels. Further, interactions between JA and other plant growth regulators make assignment of physiological roles for JA even more complicated. However, the isolation of *Arabidopsis* mutants in the JA biosynthetic pathway allow for the specific assignment of roles for JA and its precursors in plant defense or growth and development. The outcome of the interaction of plants with a given stress is governed by several factors including the genotype, the physiological state of the plant, the presence of other stress factors and environmental signals, and any specific interactions that might occur between the activated signaling pathways.

Earlier studies have assumed that plants induce SA,-JA-, and ethylene-signaling pathways that act linearly and trigger distinct plant defense responses including cell death. However, studies with various mutants of *Arabidopsis* revealed that SA-, JA- and ethylene- signaling pathways do not act independently and that plants are equipped with regulatory components to control the magnitude of each of these pathways and to mediate the interactions between these diverse signaling pathways (Genoud and Metraux 1999). The cDNA array experiments indicate that a complicated network of interactions and coordination occurs within plant defense responses. However, studies involving genotypes defective in various signaling pathways provide compelling evidence for the substantial crosstalk between JA and

other signaling pathways.

Infection of plants with certain pathogens leads to rapid necrosis and lesion formation (hypersensitive response, HR) and induction of systemic acquired resistance (SAR). Several studies confirm the role of SA for SAR induction. Other bacteria, such as those found in the rhizosphere, can promote induced systemic resistance (ISR; Pieterse et al, 1996; Thomma et al., 1998). The SA dependent SAR response is triggered by pathogens which obtain nutrients from living cells, whereas the ISR response is induced by pathogens that obtain nutrients from dead cells. ISR appears to be JA and ethylene dependent and does not require SA (ISR induction can occur in *nahG* plants). Furthermore, ISR requires *NPR1*, previously thought to be only involved in SA dependent processes (Pieterse et al, 1998). The ankyrin repeats in NPR1 could interact with different proteins from the ISR and SAR pathways to activate different responses to pathogen attack. The effects of ISR and SAR are additive and do not appear to interact negatively (van Wees et al., 2000). This observation supports the microarray data (Schenk et al., 2000) and is contrary to reports that JA and SA responses are antagonistic to each other.

Detailed studies involving various signaling mutants revealed that while JA and ethylene acts synergistically (O'Donnell et al., 1996; Penninckx et al. 1998), JA signaling, depending on plant species and stimulus, can either antagonize or synergize SA-signaling and vice versa (Dong 1998; Pieterse and van Loon 1999). However, recent studies with *Arabidopsis* protoplasts have documented that cell death induced by a fungal toxin (Fumonisin, FB1) is dependent on light and requires JA-, SA- and ethylene-signaling pathways (Asai et al., 2000). Similarly, Clarke et al., (2000) have generated a diverse array of double mutants to illustrate the interdependence and complexity of signaling pathways in inducing local and systemic resistance to pathogens.

The atmospheric pollutant ozone (O_3) also causes necrotic lesions on *Arabidopsis* leaves similar to those found during the HR found during incompatible plant pathogen interactions (for a review see Rao et al., 2000a). This similarity may result from the production of active oxygen species produced by the dissociation of O_3 in water and the "oxidative burst" during the hypersensitive cell death. Because O_3 will also induce PR proteins, it has been used to study early components of incompatible plant pathogen interactions. Similar to studies involving pathogens, researchers have identified crosstalk between JA and SA pathways in response to O_3. Rao et al. (2000b) demonstrated that JA signaling pathways attenuates SA-dependent cell death. Compared with Col-0 (an O_3 insensitive ecotype), the magnitude of O_3 induced oxidative burst and SA induced cell death in *jar1* and *fad3fad7fad8 Arabidopsis* plants was similar. Results similar to these mutants were observed in the Cvi-0, which was found to have reduced sensitivity to JA. Exogenous JA reduced the magnitude of O_3 induced lesions in Cvi-0 (Rao et al, 2000b).

Studies on lesion formation during the hypersensitive response indicate that it is composed of three separate processes, initiation, propagation, and containment. Analysis of an *Arabidopsis* mutant, *rcd1* (*radical-induced cell death 1*), indicates that whereas ethylene appears to be involved in the lesion propagation, JA functions in lesion containment (Overmyer et al., 2000). Increasing the complexity between lesion initiation, propagation and containment, studies involving various SA and ethylene overproducing mutants have demonstrated that SA is required for O_3-induced ethylene production (Rao et al., manuscript submitted). Wounding or treatment of plants with JA also reduced O_3-induced H_2O_2 levels (Schraudner et al., 1998) and cell death in O_3-sensitive plants (Orvar et al., 1997; Overmyer et al., 2000). Taken together, these studies clearly suggest that the signaling pathways of plant defense responses and cell death diverged early, and that JA-signaling constitutes an important component of anti-cell death pathways (Rao et al., 2000). However, the precise mechanism(s) by which JA-signaling regulates cell death remains to be elucidated.

Modular Switches

Examples presented above provide compelling evidence for the hypothesis that crosstalk between JA, ethylene and SA plays an important role in fine tuning complex defense responses. However, how and where different stimuli converge to result in different responses remains yet to be answered. One hypotheses put forwarded by Klessig and co-workers suggests that plants contain regulatory switches to control the temporal expression and/or the amplitude of multiple pathways. Evidence for this hypothesis comes from a *Arabidopsis* mutant, *ssi1*, which bypasses the requirement of NPR1 for the expression of PR genes and disease resistance and renders the expression of *PDF1.2* expression responsive to SA (Shah et al., 1999). PDF1.2 is a defensin whose expression is normally JA/ethylene dependent (Penninckx et al., 1996) suggesting that SSI1 functions as a molecular switch to modulate the expression of both SA- and JA-dependent pathways (Shah et al, 1999). Similarly, overexpression of a rice Ras-like G protein gene *rgp1* rendered SA accumulation and *PR1* gene expression responsive to wounding, unlike wounding of untransformed plants which activates JA-dependent signaling pathways (Sano et al., 1994).

In addition to known molecular switches such as SSI1 and G-proteins, recent studies have provided evidence for the role of a Mitogen activated protein (MAP) kinase in fine tuning the expression of JA/ethylene and SA signaling pathways (Seo et al., 1995; Petersen et al., 2000). While overexpression of a tobacco mitogen activated protein (MAP) kinase gene elevated SA and *PR1* gene expression upon wounding (Seo et al., 1995), *Arabidopsis mpk4* mutant exhibited constitutive SAR, constitutive PR gene expression, and elevated SA levels, yet induction of *PDF1.2* and *THI2.1* (thionins are basic cysteine-rich peptide with antimicrobial properties) gene expression by JA was blocked (Petersen et al., 2000). These results suggest that wildtype MPK4 suppresses SAR and acts down stream of JA in regulating the expression of *PDF1.2* and *THI1.2*. Using cDNA microarrays, Petersen et al. (2000) demonstrated that some JA induced genes did not occur in JA treated *mpk4* mutants. Because *mkp4* mutants exhibit constitutive systemic acquired resistance, Petersen et al., (2000) suggest that MKP4 may integrate JA and salicylic acid defense pathways. Other lines of evidence suggest the involvement of MAP kinases which may function to integrate the signaling pathways in the perception of external events such as wounding or other abiotic stresses (Ichimura et al, 2000; Romeis et al., 1999). However, it is not known how MPK4 can exert negative regulation of SA mediated systemic responses and positive regulation of JA-mediated defense responses. These results provide genetic evidence for both negative and positive regulatory systems that control the interaction between JA/ethylene and SA dependent pathways (Figure 4). In addition, other pathways must be involved with disease interactions because resistance to turnip crinkle virus is SA dependent yet independent of NPR, JA, and ethylene signaling pathways (Kachroo et al, 2000).

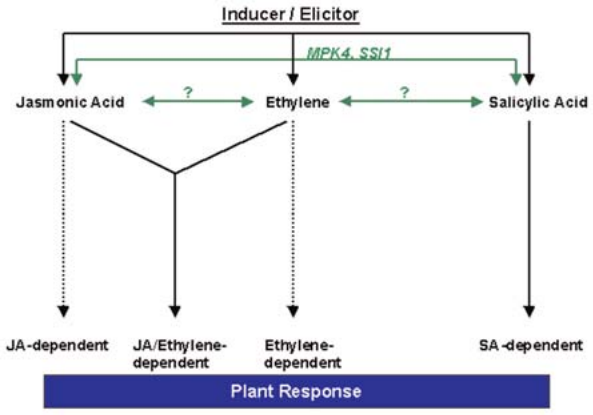

Figure 4. A hypothetical model illustrating the crosstalk between JA/ethylene and SA signaling pathways in regulating plant responses to different stress factors. Possible interactions between JA and other signaling pathways along with the known modular switches that regulate the interaction are indicated in green.

Cellular Redox State

In addition to the role of jasmonates in plant herbivory and plant pathogen interactions, there is also evidence to suggest a role for JA in regulating cellular redox state. The tripeptide g-glutamylcysteinglycine, glutathione (GSH) is the major source of non-protein thiols and plays a central role in maintaining cellular redox state and protecting plants from the onslaught of active oxygen species generated by both biotic and abiotic stress factors including heavy metals (Foyer et al., 1997). *Arabidopsis* plants treated with JA increased the induction of the steady state transcript levels of three GSH metabolic genes, *GSH1*, *GSH2* and *GR1* (Xiang and Oliver, 1998). The induction of GSH metabolic genes was found to be dependent on JA and independent of other signal molecules such as SA and hydrogen peroxide (H_2O_2). Although JA treatments alone did not alter GSH levels, JA treatments potentiated and increased GSH synthesizing capacity (Xiang and Oliver, 1998). Since GSH is a well known antioxidant metabolite known to confer stress tolerance, the demonstration that JA regulates GSH biosynthesis provides a novel role for JA in regulating cellular redox state and stress-induced responses.

Plant Herbivory

Reymond et al., (2000) also characterized the effects of herbivory on gene expression in *Arabidopsis* using Cabbage White (*Pieris rapae) and* Large White *(Pieris brassica)* caterpillars. Several genes induced by mechanical wounding were not induced by herbivory. In those cases where transcripts were induced by both treatments, in general mechanical wounding caused a greater induction. Herbivory also did not preferentially cause the induction of JA-dependent or independent genes.

Table 1. Plant Genes cited in this chapter.
The normalized public name for Arabidopsis genes comes from the January, 2002 release from TIGR/TAIR. Genes for which no normalized public name is given are from tomato.

Gene Name	Normalized Public Name.	Protein Acc.	cDNA	Reference
PLDα1	At3g15730	BAB02304.1	U36381	Qin and Wang, 2002
DAD1	At2g44810	BAB69954.1	AB060156	Ishiguro et al., 2001
SAG101	At5g14930	AAF78583.1	AF239888	He and Gan, 2002
AtVSP	At5g24780	BAA33446.1	AB006777	Utsugi et al., 1996
AtLox2	At3g45140	CAB72152.1	L23968	Bell and Mullet, 1993
AtLox1	At1g55020	AAA32827.1	L04637	Melan et al., 1993
AOS	At5g42650	CAA73184.1	Y12636	Laudert et al., 1996
HPL	AT4g15440	CAB78586.1	94J16XP (EST)	Bate et al., 1998
ADH	At1g77120	CAA54911.1	X77943	Hanfstingl et al, 1994
FAD3	At2g29980	BAA05514.1	D26508	Nishiuchi et al., 1994
FAD7	At3g11170	BAA03106.1	D14007	Iba et al., 1993
FAD8	At5g05580	AAB60302.1	U08216	Gibson et al., 1994
tomAOC		CAB95731.1	AJ272026	Ziegler et al., 2000
K13N2.10	At3g25760	NP_189204.1	NM_113475	unpublished
K13N2.11	At3g25770	NP_566776.1	NM_113476	unpublished
K13N2.12	At3g25780	NP_566777.1	NM_113477	unpublished
T6J4.4	At1g13280	NP_172786.1	NM_101199	unpublished
OPR1	At1g76680	AAC78440.1	U92460	Biesgen and Weiler, 1999
OPR2	At1g76690	AAC78441.1	U92460	Biesgen and Weiler, 1999
OPR3 (DDE1)	At2g06050	AAG15379.1	AF293653	Stintzi and Browse, 2000
AIM1	At4g29010	AAD18041.1	AF123253	Richmond and Bleecker, 1999
PED1	At2g33150	BAA25249.1	AB008855	Hayashi et al., 1998
PKT1	At5g48880	AAC19122.1	AF062590	Rocha et al., 1996
PKT2	At5g48880	AAC23571.1	AF062591	Rocha et al., 1996
JMT	At1g19640	AAG23343.1	AY008434	Seo et al., 2001
COI1	At2g39940	AAC17498.1	AF036340	Xie et al., 1998
TIR1	At3g62980	AAB69176.1	AF005048	Ruegger et al., 1998
E8		CAA31789.1	X13437	Deikman and Fischer. (1988).
GBF3	At2g46270	CAA45358.1	X63896	Schindler et al., 1992
RAP2.6	At1g43160	AAC49772.1	AF003099	Okamuro et al., 1997
NPR1	At1g64280	AAC49611.1	U76707	Cao et al., 1997
AtERF1	At4g17500	BAA32418.1	AB008103	Fujimoto et al., 2000
AtERF2	At5g47220	BAA32419.1	AB008104	Fujimoto et al., 2000
CAD2-1	At4g23100	AAD14544.1	AF068299	Cobbet et al., 1998
PDF1.2	At5g44420		A68653	Broekaert et al., 1998
PR1	At2g19990	AAA32841.1	M59196	Metzler and Klessig, 1991
MPK4	At4g01370	AAK64089.1	AY040031	
THI2.1	At1g72260	AAC41678.1	L41244	Epple et al., 1995
GSH1	At4g23100	CAA82626.1	Z29490	May and Leaver, 1994
GSH2	At5g27380	AAA99146.1	U53856	Wang and Oliver, 1966
GR1	At3g24170	AAB67841.1	U37697	Loebler, 1996

Plant Reproduction

In addition to a role in plant defense, oxylipins also appear to play a role in plant reproduction. For example, exogenous JA decreases the photosynthetic rate of plants and causes a reduction in bud formation (Barendse et al., 1985). Dicot flowers contain high levels of jasmonates including amino acid conjugates or amides (Miersch et al., 1997) and it has been speculated that a tissue specific oxylipin signature might regulate flowering processes in plants. The ultimate role of JA in flower development is demonstrated by the observation that mutants that have defects in JA biosynthesis or perception have defects in either flower development or male sterility (Feys et al., 1994; Ishiguro et al., 2001; McConn and Browse, 1996; Sanders et al., 2000; Stintzi and Browse, 2000). Furthermore, Capella et al., (2001) have demonstrated that COI1-dependent myrosinase activity controls the expression of flower-specific myrosinase-binding protein homologs. A critical requirement for JA but not OPDA in

pollen development was identified (McConn and Browse, 1996; Stintzi and Browse, 2000). It has been hypothesized that JA synthesized in filaments regulates the water transport in stamens and petals. Studies with the *Arabidopsis* mutant *defective in anther dehiscence* have shown that *DAD1* gene regulated JA biosynthesis is important for the synchronization of pollen maturation, anther dehiscence and flower opening (Ishiguro et al., 2001).

Analysis of Arabidopsis late-flowering mutants *fca-1* and *cad2-1* with defects in GSH biosynthesis treated with exogenous GSH or GSH inhibitors revealed a role for GSH in promoting flowering (Ogawa et al., 2001). Since some of the JA deficient or sensitivity mutants have defects in flowering and GSH production requires JA, it is possible that JA-dependent GSH production may play an important role in plant reproduction. Studies showing that many of the abiotic stress factors induce the biosynthesis of several biologically active oxylipins and promote flowering (Yokoyama et al., 2000) support the above hypothesis. However, detailed studies are required to understand how plants integrate signals emanating from different molecules and instigate a complex physiological response such as flowering and reproduction.

Leaf Senescence

In a impressive series of experiments, He et al. (2002) investigated the role of JA in leaf senescence. Exogenous JA caused senescence in attached and detached leaves in wild-type leaves of Arabidopsis. Senescence was not induced in *coi1* plants, suggesting that the JA signaling pathway is required for the JA promotion of leaf senescence. Furthermore, JA levels in senescing leaves were 4 fold higher than in green, non-senescing leaves. Concurrent with this increase in JA levels, genes encoding enzymes that catalyze most of the reactions of the JA biosynthetic pathway were differentially activated during leaf senescence in Arabidopsis (He et al., 2000). These data suggest that JA may play a role in leaf senescence. Senescence is believed to caused by the expression of senescence associated genes (SAGs; Bleecker and Patterson, 1997; Buchanan-Wollaston, 1997; Gan and Amasino, 1997; Hajouj et al., 2000; Quirino et al., 2000). JA could cause leaf senescence by inducing the expression of (SAGs; Pathier, 1990). It is known that JA can induce a subset of SAGs (Park et al., 1998; Kinoshita et al., 1999; Schenk et al., 2000).

Other Oxylipins

Many of the known biotic and abiotic stress factors initiate membrane peroxidation and elicit the production of jasmonates and other biologically active oxylipins (Reymond and Farmer, 1998). Historically, JA is the major oxylipin that has received wide attention from ecologists and stress physiologists alike. However, recent studies increasingly suggest important roles for other oxylipins in regulating plant defense responses. Four lipid degrading enzymes, phospholipase D, phosphatidic acid phosphatase, lypolytic acyl hydrolase and lipoxygenases (LOX) are attributed to increase the biosynthesis of various lipid-based signaling molecules believed to participate in plant stress responses (Bate and Rothstein, 1998; Thompson et al., 1998; Hamberg et al., 1999; Wang, 1999; Schaller, 2001; Howe and Schilmiller, 2002).

Among the four lipid degrading enzymes, LOX mediated lipid peroxidation leads to products such as polyunsaturated fattyacids (PUFAs), alkenals and aldehydes all believed to participate in plant stress responses (Bate and Rothstein, 1998; Schaller, 2001). In plants, based on their affinity to introduce molecular oxygen into linoleic acid or linolenic acid, two types of LOXs, 9- or 13-, were identified. Functional analyses using transgenic plants expressing different LOXs have revealed the role of LOX-dependent products in regulating plant defense responses. Arabidopsis plants with decreased AtLox2 do not show an increase in JA levels and *AtVSP* transcripts in response to wounding (Bell et al., 1995). Similarly Arabidopsis plants with decreased linolenic acid were found to be susceptible to insect attack (McConn et al., 1997). Tobacco plants accumulated 9-hydroperoxy PUFAs after the initiation of hypersensitive response in response to cryptogein treatments (Rusterucci et al., 1998). Further, treatment of plants with 13-hydroperoxy-9,11(*Z*,E)-octadecadienoic acid induced necrotic lesions suggesting that LOX-mediated intermediates are important components of plant defense responses in response to pathogen attack. More work is needed to completely understand the role of lipid hydroperoxides including C6 volatiles in regulating plant defense responses and their interaction with other signaling pathways.

CONCLUDING REMARKS

The volume of publications and reviews on jasmonate

since 1997 documents the increasing interest in this compound and its role in plants. Research on this topic has solidified our understanding of the chemistry and biosynthetic pathways for oxylipin production. However, additional research is needed into the mechanisms that regulate the synthesis of oxylipins in plants during development and in response to wounding and other stress factors. Further knowledge is also needed as to the role of the various oxylipins and their changing composition in different tissues during growth and development and during pathogen or insect attack. Mutants deficient in JA or its precursors or with altered perception of JA provided definitive evidence on the role of oxylipins in plant growth and development in *Arabidopsis*. As we notice a higher level of complexity in the signaling network, more detailed information on pathways and networks of crosstalk is required to comprehensively understand signal processing. Plants may possess regulatory genes that can trigger multiple signaling pathways appropriately as and when needed. Our understanding of the JA signal transduction pathway is advancing rapidly as genes identified through cDNA microarrays are analyzed and through the analysis of JA insensitive mutants. Further, development of various novel double and/or triple mutants defective in combinations of various signaling pathways, and the use of DNA chips to identify the gene circuits associated with the signaling network should help us develop powerful models to represent the extensive cross talk between various signaling networks. Insight gained from these studies should lead to better design of durable plant defense and improved utilization of proteins and genes from non-plant sources for plant protection.

ACKNOWLEDGEMENTS

Due to the availability of other reviews, we apologize in advance for being unable to cite all of the excellent publications on this topic.

REFERENCES

Asai, T., Stone, J.M., Heard, J.E., Kovtun, Y., Yorgey, P., Sheen, J. and Ausubel, F.M. (2000). Fumonisin B1-induced cell death in *Arabidopsis* protoplasts requires Jasmonate-, Ethylene-, and Salicylate-dependent signaling pathways. Plant Cell **12**, 1823-1835.

Bai, C., Sen, P., Hofmann, K., Ma, L., Goebl, M., Harper, J.W. and Elledge S.J. (1996). SKP1 connects cell cycle regulators to the ubiquitin proteolysis machinery though a novel motif, the f-box. Cell **86**, 263-274.

Baldwin, I.T. (1999). The jasmonate cascade and the complexity of induced defense against herbivore attack. In The role of plant secondary metabolites and their utilization in biotechnology: M. Wink, ed, (Sheffield: Sheffield Academic Press), pp. 155-186.

Baldwin, I.T., Zhang, Z-P., Diab, N., Ohnmeiss, T.E., McCloud E.S., Lynds, G.Y. and E. A. Schmelz. (1997). Quantification, correlations and manipulations of wound-induced changes in jasmonic acid and nicotine in *Nicotiana sylvestris*. Planta **201**, 397-404.

Barendse, G.W.M., Croes, A.F., van den Ende, G., Bosveld, M. and Creemers, T. (1985). Role of hormones on flower bud formation in thin layer explants of tobacco. Biol. Plant. **27**, 408-412.

Bate, N.J., Riley, J.C.M., Thompson, J.E. and Rothstein, S.J. (1998). Quantitative and qualitative differences in C-6 volatile production from the lipoxygenase pathway in an alcohol dehydrogenase mutant of *Arabidopsis thaliana*. Physiol. Plant., **104**, 97-104.

Bate, N.J. and Rothstein, S.J. (1998). C6-volatiles derived from the lipoxygenase pathway induce a subset of defense-related genes. Plant J. **16**, 561-569.

Bate, N.J., Sivasankar, S., Moxon, C., Riley, J.M., Thompson, J.E. and Rothstein, S.J. (1998). Molecular characterization of an *Arabidopsis* gene encoding hydroperoxide lyase, a cytochrome P-450 that is wound inducible. Plant Physiol. **117**, 1393-400.

Bell, E. and Mullet, J. E. (1993). Characterization of an *Arabidopsis* lipoxygenase gene responsive to methyl jasmonate and wounding. Plant Physiol. **103**, 1133-1137.

Bell, E., Creelman, R. A. and Mullet, J. E. (1995). A chloroplast lipoxygenase is required for wound-induced jasmonic acid accumulation in *Arabidopsis.* Proc. Natl. Acad. Sci. USA **92**, 8675-8679.

Benedetti, C. E., Xie, D. and Turner, J. G. (1995). *COI1*-dependent expression of an *Arabidopsis* vegetative storage protein in flowers and siliques and in response to coronatine or methyl jasmonate. Plant Physiol. **109**, 567-572.

Berger, S., Bell, E. and Mullet, J. E. (1996). Two methyl jasmonate-insensitive mutants show altered expression of *AtVSP* in response to methyl jasmonate and wounding. Plant Physiol. **111**, 525-531

Biesgen, C. and Weiler, E.W. (1999). Structure and regulation of *OPR1* and *OPR2*, two closely related genes encoding 12-oxophytodienoic acid-10,11-reductases from *Arabidopsis thaliana*. Planta **208**, 155-165.

Blechert, S., Brodschelm, W., Holder, S., Kammerer, L., Kutchan, T.M., Mueller, M.J., Xia, Z. and Zenk, M.H. (1995). The octadecanoic pathway: Signal molecules for the regulation of secondary pathways. Proc. Natl. Acad. Sci. USA **92**, 4099-4105.

Bleé, E. and Joyard, J. (1996). Envelope membranes from spinach chloroplasts are a site of metabolism of fatty acid hydroperoxides. Plant Physiol. **110**, 445-454

Blée, E. and Schuber, F. (1995). Oxylipins in plants: The peroxygenase pathway. In Plant lipid metabolism, J.-C. Kader and P. Mazliak, eds (Dordrecht: Kluwer Academic Publishers), pp. 262-264.

Bleecker, A.B. and Patterson, S.E. (1997) Last exit: senescence, abscission, and meristem arrest in Arabidopsis. Plant Cell **9,** 1169-1179.

Broekaert, W.F., Thomma, B.P., Penninckx, I.A., Terras, F.R., Manners, J.M. and Kazan, K. (1998). Plant protection method. Patent: WO 9800023-A 21.

Bruckner, C. Kramell, R., Schneider, G., Schmidt, J., Priess, A., Sembdner, G. and Schreiber, K. (1988). N-[(-)-jasmonyl]-S-tryptophan and a related tryptophan conjugate from *Vicia faba*. Phytochemistry **27**, 275-276.

Buchanan-Wollaston, V. (1997) The molecular biology of leaf senescence. J Exp Bot **48**, 181-199.

Capella, A.N., Menossi, M., Arruda, P. and Benedetti, C.E. (2001). COI1 affects myrosinase activity and controls the expression of two flower-specific myrosinase-binding protein homologues in *Arabidopsis*. Planta, **213**, 691-699.

Chen, W., Provart, N.J., Glazebrook, J., Katagiri, F., Chang, H-S., Eulgem, T., Mauch, F., Luan, S., Zou, G., Whitman, S.A., Budworth, P.R., Tao, Y., Xie, Z., Chen, X., Lam, S., Kreps, J.A., Harper, J.F., Si-Ammour, A., Mauch-Mani, B., Heinlein, M., Kobayashi, K., Hohn, T., Dangl, J.L., Wang, X. and Zhu, T. (2002). Expression profile matrix of Arabidopsis transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell, **14**, 559-574.

Clarke, J.D., Liu, Y., Klessig, D.F. and Dong, X. (1998). Uncoupling PR gene expression from NPR1 and bacterial resistance: characterization of the dominant *Arabidopsis cpr 6-1* mutant. Plant Cell **10**, 557-69.

Cao, H., Glazebrook, J., Clarke, J.D., Volko, S. and Dong, X. (1997). The Arabidopsis NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell **88**, 57-63 (1997)

Cobbett, C.S., May, M.J., Howden, R. and Rolls, B. (1998). The glutathione-deficient, cadmium-sensitive mutant, cad2-1, of Arabidopsis thaliana is deficient in gamma-glutamylcysteine synthetase. Plant J. **16**, 73-78

Conconi, A., Miquel, M., Browse, J.A. and Ryan, C.A. (1996). Intracellular levels of free linolenic and linoleic acids increase in tomato leaves in response to wounding. Plant Physiol. **111**, 797-803.

Creelman, R.A. (1999). Jasmonate perception: characterization of COI1 mutants provides the first clues. Trends Plant Sci. **10**, 367-368.

Creelman, R.A. and Mullet J.E. (1995). Jasmonic acid distribution and action in plants: Regulation during development and response to biotic and abiotic stress. Proc. Natl. Acad. Sci. USA **92**, 4114-4119.

Creelman, R.A. and Mullet, J.E. (1997). Biosynthesis and action of jasmonates in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. **48**, 355-381

Creelman, R. A., Tierney, M. L. and Mullet, J. E. (1992). Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene expression. Proc. Nati. Acad. Sci. USA **89**,4938-4941.

Dhondt, S., Geoffroy, P., Stelmach, B.A., Legrand, M. and Heitz, T. (2000). Soluble phospholipase A2 activity is induced before oxylipin accumulation in tobacco mosaic virus-infected tobacco leaves and is contributed by patatin-like enzymes. Plant J. **23**, 431-440.

Deikman, J. and Fischer, R.L. (1988). Interaction of a DNA binding factor with the 5'-flanking region of an ethylene-responsive fruit ripening gene from tomato. EMBO J. **7**, 3315-3320

Dong, X. (1998). SA, JA, ethylene, and disease resistance in plants. Curr. Opin. Plant Biol. **1**, 316-323..

Doares, S.H., Narvaez-Vasquez, J., Conconi, A. and Ryan, C.A. (1995). Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. Plant Physiol. **108,** 1741-1746.

Ellis, C. and Turner, J.G. (2001). The *Arabidopsis* mutant *cev1* has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. Plant Cell **13**, 1025-1033.

Epple, P., Apel, K. and Bohlmann, H. (1995). An *Arabidopsis thaliana* thionin gene is inducible via a signal transduction pathway different from that for pathogenesis-related proteins. Plant Physiol. **109**, 813-820.

Farmer, E. E. and Ryan, C. A. (1992). Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell **4**, 129-134.

Farmer, E.E., Weber, H. and Vollenweider, S. (1998). Fatty acid signaling in *Arabidopsis*. Planta **206,** 167-174.

Feys, B. J. F., Benedetti, C. E., Penfold, C. N. and Turner, J. G. (1994). *Arabidopsis* mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. Plant Cell **6**, 751-759.

Foyer, C.H., Lopez-Delgado, H., Dat, J.F. and Scott, I.M. (1997). Hydrogen peroxide- and glutathione-associated mechanisms of acclimatory stress tolerance and signaling. Physiol. Plant. **100**, 241-254.

Fujimoto, S.Y., Ohta, M., Usui, A., Shinshi, H. and Ohme-Takagi, M. (2000). Arabidopsis ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression. Plant Cell **12**, 393-404

Gan, S. and Amasino, R. (1997) Making sense of senescence: molecular genetic regulation and manipulation of leaf senescence. Plant Physiol **113**, 313-319.

Gerhardt, B. (1992). Fatty acid degradation in plants. Prog. Lipid Res. **31**, 417-446

Genoud, T. and Metraux, J-P. (1999). Crosstalk in plant cell signaling: structure and function of the genetic network. Trends Plant Sci. **4**, 503-507.

Gibson, S., Arondel, V., Iba, K. and Somerville, C. 1994. Cloning of a temperature-regulated gene encoding a chloroplast omega-3 desaturase from Arabidopsis thaliana. Plant Physiol. **106**, 1615-1621.

Gundlach, H., Müller, M. J., Kutchan, T. M. and Zenk, M. H. (1992). Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. Proc. Natl. Acad. Sci. USA **89**, 2389-2393.

Gundlach, H. and Zenk, M.H. (1998). Biological activity and biosynthesis of pentacyclic oxylipins: The linoleic acid pathway. Phytochemistry **47**, 527-537.

Hajouj, T., Michelis, R. and Gepstein, S. (2000) Cloning and characterization of a receptor-like protein kinase gene associated with senescence. Plant Physiol **124**, 1305-1314.

He, Y., Fukushige, H., Hildebrand, D.F. and Gan, S. (2002). Evidence supporting a role of jasmonic Acid in Arabidopsis leaf senescence. Plant Physiol.**128**, 876-84.

He. Y. and Gan, S. (2002). A gene encoding an acyl hydrolase is involved in leaf senescence in Arabidopsis. Plant Cell.**14**, 805-15.

Hamberg, M. and Gardner H. W. (1992). Oxylipin pathway to jasmonates: biochemistry and biological significance. Biochim. Biophys. Acta **1165**, 1-18.

Hamberg, M., Sanz, A. and Castresana, C. (1999). a-oxidation of fatty acids in higher plants. Identification of a pathogen-induced oxygenase (PIOX) as an a-dioxygenase and biosynthesis of 2-hydroperoxylinolenic acid. J. Biol. Chem. **274**, 24503-24513.

Harms, K., Atzorn, R., Brash, A., Kühn, H., Wasternack, C., Willmitzer, L. and Peña-Cortés, H. (1995). Expression of a flax allene oxide synthase cDNA leads to increased endogenous jasmonic acid (JA) levels in transgenic potato plants but not to a corresponding activation of JA-responding genes. Plant Cell **7**, 1645-1654.

Hanfstingl, U., Berry, A., Kellogg, E.A., Costa, J.T. III, Rudiger, W. and Ausubel, F.M. (1994). Haplotypic divergence coupled with lack of diversity at the *Arabidopsis thaliana* alcohol dehydrogenase locus: roles for both balancing and directional selection? Genetics **138**, 811-828.

Hause, B., Stenzel, I., Miersch, O., Maucher, H., Kramell, R., Ziegler, J. and Wasternack, C. (2000). Tissue-specific oxylipin signature of tomato flowers: Allene oxide cyclase is highly expressed in distinct flower organs and vascular bundles. Plant J. **24**, 113-126.

Hayashi, M., Aoki, M., Kato, A., Kondo, M. and Nishimura, M. (1996). Transport of chimeric proteins contain a carboxy terminal targeting signal into plant microbodies. Plant J. **10**, 225-234.

Hayashi, M., Toriyama, K., Kondo, M., Nishimura, M. (1998) 2,4-Dichlorophenoxybutyric acid-resistant mutants of *Arabidopsis* have defects in glyoxysomal fatty acid beta-oxidation. Plant Cell **10**, 183-195

von Heijne, G., Steppuhn, J. and Herrmann, R.G. (1989). Domain structure of mitochondrial and chloroplast targeting peptides. Eur. J. Biochem. **180**, 535-545.

Hilpert, B., Bohlmann, H., op den Camp, R.O., Przybyla, D., Miersch, O., Buchala, A. and Apel, K. (2001). Isolation and characterization of signal transduction mutants of Arabidopsis thaliana that constitutively activate the octadecanoid pathway and form necrotic microlesions. Plant J. **26**, 435-46.

Howe, G.A. and Schilmiller, A.L. (2002). Oxylipin metabolism in response to stress. Curr. Opin. Plant Biol. **5**, 230-236.

Iba, K., Gibson, S., Nishiuchi, T., Fuse, T., Nishimura, M., Arondel, V., Hugly, S. and Somerville, C. (1993). A gene encoding a chloroplast omega-3 fatty acid desaturase complements alterations in fatty acid desaturation and chloroplast copy number of the fad7 mutant of *Arabidopsis thaliana*. J. Biol. Chem. **268**, 24099-24105.

Ichimura, K., Mizoguchi, T., Yoshida, R., Yuasa, T. and Shinozaki, K. (2000). Various abiotic stresses rapidly activate *Arabidopsis* MAP kinases ATMPK4 and ATMPK6. Plant J. **24**, 655-665.

Ishiguro S., Kawai-Oda, A., Ueda, J., Nishida, I. and Kiyotaka Okada, K. (2001). The *DEFECTIVE IN ANTHER DEHISCENCE1* gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in *Arabidopsis*. Plant Cell **13,** 2191-2209.

Jensen, A.B., Raventos, D. and Mundy, J. (2002). Fusion genetic analysis of jasmonate-signalling mutants in *Arabidopsis*. Plant J. **29**, 595-606.

Kachroo, P., Yoshioka, K., Shah, J., Dooner, H.K. and Klessig, D.F. (2000). Resistance to turnip crinkle virus in *Arabidopsis* is regulated by two host genes and is salicylic acid dependent but NPR1, ethylene, and jasmonate independent. Plant Cell **12**, 677-690.

Kinoshita, T., Yamada, K., Hiraiwa, N., Kondo, M., Nishimura, M. and Hara-Nishimura, I. (1999) Vacuolar processing enzyme is up-regulated in the lytic vacuoles of vegetative tissues during senescence and under various stressed conditions. Plant J **19**, 43-53.

Kliebenstein, D.J., Figuth, A. and Mitchell-Olds, T. (2002). Natural variation of insect herbivory related signal transduction in Arabidopsis – The identification of multiple Methyl Jasmonate signal transduction pathways. Key Stone Symposia on "Specificity and Crosstalk in Plant Signal Transduction," January 22-27, 2002, Tahoe City, CA, pp. 57.

Kneissl, M.L. and Deikman, J. (1996). The tomato E8 gene influences ethylene biosynthesis in fruit but not in flowers. Plant Physiol. **112**, 537-547.

Kobe, B. and Deisenhofer, J. (1994). The leucine-rich repeat: a versatile binding motif. Trends Biol. Sci. **19**,415-421.

Kramell, R., Atzorn, R., Schneider, G., Miersch, O., Brückner, C., Schmidt, J., Sembdner, G. and Parthier, B. (1995). Occurrence and identification of jasmonic acid and its amino acid conjugates induced by osmotic stress in barley leaf tissue. J. Plant Growth Regul. **14**, 29-36.

Kramell, R., Miersch, O., Atzorn, R., Parthier, B. and Wasternack, C. (2000). Octadecanoid-derived alteration of gene expression and the "oxylipin signature" in stressed barley leaves. implications for different signaling pathways. Plant Physiol. 2000 **123**, 177-188.

Kubigsteltig, I., Laudert, D. and Weiler, E.W. (1999). Structure and regulation of the *Arabidopsis* thaliana allene oxide synthase. Planta **208**, 463-71.

Laudert, D., Hennig, P., Stelmach, B.A., Muller, A., Andert, L. and Weiler, E.W. (1997). Analysis of 12-oxo-phytodienoic acid enantiomers in biological samples by capillary gas chromatography-mass spectrometry using cyclodextrin stationary phases. Anal. Biochem. **246**, 211-217.

Laudert, D., Pfannschmidt, U., Lottspeich, F., Hollander-Czytko, H. and Weiler, E.W. (1996). Cloning, molecular and functional characterization of *Arabidopsis thaliana* allene oxide synthase (CYP 74), the first enzyme of the octadecanoid pathway to jasmonates. Plant. Mol. Biol. **31**, 323-335.

Laudert, D., Schaller, F. and Weiler, E.W. (2000). Transgenic *Nicotiana tabacum* and *Arabidopsis thaliana* plants overexpressing allene oxide synthase. Planta. **211**, 163-165.

Laudert, D. and Weiler, E.W. (1998). Allene oxide synthase: A major control point in *Arabidopsis thaliana* octadecanoid signaling. Plant J. **15**, 675-684.

Lee, S., Suh, S., Kim, S., Crain, R.C., Kwak, J.M., Nam, H.G. and Lee, Y. (1997). Systemic elevation of phosphatidic acid and lysophospholipid levels in wounded plants. Plant J. **12**, 547-556.

Leon, J., Rojo, E., Titarenko, E. and Sanchez-Serrano, J.J. (1998). Jasmonic acid-dependent and -independent wound signal transduction pathways are differentially regulated by Ca2+/calmodulin in *Arabidopsis thaliana*. Mol. Gen. Genet. **258**, 412-419.

Li, F.N. and Johnston, M. (1997). Grr1 of *Saccharomyces cerevisiae* is connected to the ubiquitin proteolysis machinery through Skp1: coupling glucose sensing to gene expression and the cell cycle. EMBO J. **16**, 5629-5638.

Loebler, M. (1996). An Arabidopsis cDNA (Accession No. U37697) homologous to glutathione reductase (PGR96-053). Plant Physiol. **111**, 1353.

Matsui, K., Wilkinson, J., Hiatt, B., Knauf, V. and Kajiwara, T. (1999). Molecular cloning and expression of *Arabidopsis* fatty acid hydroperoxide lyase. Plant Cell Physiol. **40**, 477-481.

May, M.J. and Leaver, C.J. (1994). *Arabidopsis thaliana* gamma-glutamylcysteine synthetase is structurally unrelated to mammalian, yeast, and *Escherichia coli* homologs. Proc. Natl. Acad. Sci. U.S.A. **91**, 10059-10063.

McConn, M. and Browse, J. (1996). The critical requirement for linolenic acid is pollen development, not photosynthesis, in an *Arabidopsis* mutant. Plant Cell **8**, 403-416.

McConn, M., Creelman, R.A, Bell E., Mullet, J.E. and Browse J. (1997). Jasmonate plays an essential role in plant insect defense. Proc. Natl. Acad. Sci. USA. **94**, 5473-5477.

Melan, M. A., Dong, X., Endara, M. E., Davis, K. R., Peterman, T. K. and Ausubel, F. M. (1993). An *Arabidopsis thaliana* lipoxygenase gene can be induced by pathogens, abscisic acid, and methyl jasmonate. Plant Physiol. **101**, 441-450.

Metzler, M.C., Cutt, J.R. and Klessig, D.F. (1991). Isolation and characterization of a gene encoding a PR-1-like protein from *Arabidopsis thaliana.* Plant Physiol. **96**, 346-348.

Miersch, O., Knofel, H.D., Schmidt, J., Kramell, R. and Parthier, B. (1997). A jasmonic acid conjugate *N*-[(-)-jasmonoyl]-tyramine from *Petunia* pollen. Phytochem. **47**, 327-329.

Mueller, M. J. and Brodschelm, W. (1994). Quantification of jasmonic acid by capillary gas chromatography-negative chemical ionization-mass spectrometry. Anal. Biochem. **218**, 425-435.

Mussig, C., Biesgen, C., Lisso, J., Uwer, U., Weiler E.W. and Altmann, T. (2000). A novel stress-inducible 12-oxophytodienoate reductase from *Arabidopsis thaliana* provides a potential link between brassinosteroid action and jasmonic acid synthesis. J. Plant Physiol. **157**, 143-152.

Narvaez-Vasquez, J., Florin-Christensen, J. and Ryan, C.A. (1999). Positional specificity of a phospholipase A activity induced by wounding, systemin, and oligosaccharide elicitors in tomato leaves. Plant Cell. **11**, 2249-2260.

Niki, T., Mitsuhara, I., Seo, S., Ohtsubo, N. and Ohashi, Y. (1998). Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. *Plant Cell Physiol*. **39,** 500-507.

Nishiuchi, T., Nishimura, M., Arondel, V. and Iba, K. (1994). Genomic nucleotide sequence of a gene encoding a microsomal omega-3 fatty acid desaturase from *Arabidopsis thaliana*. Plant Physiol. **105**, 767-768.

O'Donnell, P.J.O., Calvert, C., Atzorn, R., Wasternack, C., Leyser, H.M.O. and Bowles, D.J. (1996). Ethylene as a signal mediating the wound response of tomato plants. Science **274**, 1914-1917.

Ogawa, K., Tasaka, Y., Mino, M., Tanaka, Y. and Iwabuchi, M. (2001). Association of glutathione with flowering in *Arabidopsis thaliana*. Plant Cell Physiol. **42**, 524-530.

Okamuro, J.K., Caster, B., Villarroel, R., Van Montagu, M. and Jofuku, K.D. (1997). The AP2 domain of APETALA2 defines a large new family of DNA binding proteins in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A. **94**, 7076-7081

Orvar, B.L., McPherson, J. and Ellis, B.E. (1997). Pre-activating wounding response in tobacco prior to high-level ozone exposure prevents necrotic injury. Plant J. **11**, 203-212.

Overmyer, K., Tuominen, H., Kettunen, R., Betz, C., Langebartels, C., Sandermann, H. Jr. and Kangasjarvi, J. (2000). Ozone-sensitive *Arabidopsis rcd1* mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. Plant Cell 12, 1849-1862.

Park, J., Oh, S., Kim, Y., Woo, H. and Nam, H. (1998) Differential expression of senescence-associated mRNAs during leaf senescence induced by different senescence-inducing factors in Arabidopsis. Plant Mol Biol **37**, 445-454.

Parthier, B. (1990) Jasmonates: hormonal regulators or stress factors in leaf senescence? J Plant Growth Regul 9, 57-63

Pena-cortes, H., Albrecht, T., Prat, S., Weiler, E.W. and Willmitzer, L. (1993). Aspirin prevents wound-induced genre expression in tomato leaves by blocking jasmonic acid biosynthesis. *Planta* **191,** 123-128.

Petersen, M., Brodersen, P., Naested, H., Andreasson, E., Lindhart, U., Johansen, B., Nielsen, H.B., Lacy, M., Austin, M.J., Parker, J.E., Sharma, S.B., Klessig, D.F., Martienssen, R., Mattsson, O., Jensen, A.B. and Mundy, J. (2000). *Arabidopsis* MAP kinase 4 negatively regulates systemic acquired resistance. Cell **103**, 1111-1120.

Penninckx, I.A., Eggermont, K., Terras, F.R., Thomma, B.P., De Samblanx, G.W., Buchala, A., Metraux, J.P., Manners, J.M. and Broekaert, W.F. (1996). Pathogen-induced systemic activation of a plant defensin gene in *Arabidopsis* follows a salicylic acid-independent pathway. Plant Cell **8**, 2309-2323.

Penninckx, I.A.M.A., Thomma, B.P.H.J., Buchala, A., Metraux, J.-P., and Broekaert, W.F. (1998). Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. *Plant Cell*, **10**, 2103-2113.

Pieterse, C.M., van Wees, S.C., Hoffland, E., van Pelt, J.A. and van Loon L.C. (1996). Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. Plant Cell **8**, 1225-1237.

Pieterse, C.M., van Wees, S.C., van Pelt, J.A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P.J. and van Loon, L.C. (1998). A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. Plant Cell **10**, 1571-1580.

Pieterse, C.J.M. and van Loon, L.C. (1999). Salicylic acid-independent plant defense pathways. Trends Plant Sci **4**, 52-58.

Qin, W., Pappan, K. and Wang, X. (1997). Molecular heterogeneity of phospholipase D (PLD). Cloning of PLDg and regulation of plant PLDγ, -β, and -α by polyphosphoinositides and calcium. J Biol. Chem. **272**, 28267-28273.

Qin, C. and Wang, X. (2002) The Arabidopsis Phospholipase D Family. Characterization of a Calcium-Independent and Phosphatidylcholine-Selective PLDz1 with Distinct Regulatory Domains. Plant Physiol.**128**, 1057-68.

Quirino, B.F., Noh, Y.S., Himelblau, E. and Amasino, R.M. (2000) Molecular aspects of leaf senescence. Trends Plant Sci 5, 278-282

Rao, M.V. and Davis, K.R. (1999). Ozone-induced cell death ocurs via two distinct mechanisms in *Arabidopsis*: the role of salicylic acid. Plant J. **17**, 603-614.

Rao, M.V., Koch, J.R. and Davis, K.R. (2000a). Ozone: a tool for probing programmed cell death in plants. Plant Mol. Biol.. **44**, 345-358.

Rao, M.V., Lee, H., Creelman, R.A., Mullet, J.E. and Davis, K.R. (2000b). Jasmonic acid signaling modulates ozone-induced hypersensitive cell death. Plant Cell **12**, 1633-1646.

Riechmann, J.L., Heard, J., Martin, G., Reuber, L., Jiang, C.Z., Keddie, J., Adam, L., Pineda, O., Ratcliffe, O.J., Samaha, R.R., Creelman, R.L., Pilgrim, M., Broun, P., Zhang, J.Z., Ghandehari, D., Sherman, B.K. and Yu, G.L. (2000). Arabidopsis transcription factors: Genome-wide comparative analysis among eukaryotes. Science, **290**, 2105-2110.

Reymond P. and Farmer E.E. (1998). Jasmonate and salicylate as global signals for defense gene expression. Curr. Opin. Plant Biol.**1**, 404-411.

Reymond, P., Weber, H., Damond, M. and Farmer, E.E. (2000). Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. Plant Cell. **12**, 707-720.

Richmond, T.A. and Bleecker, A.B. (1999). A Defect in ß-Oxidation Causes Abnormal Inflorescence Development in *Arabidopsis*. Plant Cell **11**, 1911-1924.

Romeis, T., Piedras, P., Zhang, S., Klessig, D.F., Hirt, H. and Jones J.D. (1999). Rapid Avr9- and Cf-9 -dependent activation of MAP kinases in tobacco cell cultures and leaves: convergence of resistance gene, elicitor, wound, and salicylate responses. Plant Cell **11**, 273-287

Rocha, P.S.C., Topping, J.F. and Lindsey, K. (1996) Promoter trapping in Arabidopsis: one T-DNA tag, three transcripts and two thiolase genes. J Exp Bot **47**, ss24.

Rojo, E., Titarenko, E., Leon, J., Berger, S., Vancanneyt, G. and Sanchez-Serrano, J.J. (1998). Reversible protein phosphorylation regulates jasmonic acid-dependent and -independent wound signal transduction pathways in *Arabidopsis thaliana*. Plant J. **13**, 153-165.

Ruegger, M., Dewey, E., Gray, W.M., Hobbie, L., Turner, J. and Estelle, M. (1998). The TIR1 protein of *Arabidopsis* functions in auxin response and is related to human SKP2 and yeast grr1p. Genes and Dev. **12**, 198-207.

Rusterucci, C., Montillet, J.L., Agnel, J.P., Battesti, C., Alonso, B., Knoll, A., Bessoule, J.J., Etienne, P., Suty, L., Blein, J.P. and Triantaphylides, C. (1999). Involvement of lipoxygenase-dependent production of fatty acid hydroperoxides in the development of the hypersensitive cell induced by cryptogein on tobacco leaves. J. Biol. Chem. **274**, 36446-36455.

Ryu, S.B. and Wang, X. (1998). Increase in free linolenic and linoleic acids associated with phospholipase D-mediated hydrolysis of phospholipids in wounded castor bean leaves. Biochim. Biophys. Acta. **1393**, 193-202.

Sanders, P.M., Lee, P.Y., Biesgen, C., Boone, J.D., Beals, T.P., Weiler, E.W. and Goldberg R.B. (2000). The *Arabidopsis DELAYED DEHISCENCE1* Gene Encodes an Enzyme in the Jasmonic Acid Synthesis Pathway. Plant Cell **12**, 1041-1062.

Sano, H., Seo, S., Orudgev, E., Youssefian, S., Ishizuka, K. and Ohashi, Y. (1994). Expression of the gene for a small GTP binding protein in transgenic tobacco elevates endogenous cytokinin levels, abnormally induces salicylic acid in response to wounding, and increases resistance to tobacco mosaic virus infection. Proc. Natl. Acad. Sci. USA, **91**, 10556-10560.

Sasaki, Y., Asamizu, E., Shibata, D., Nakamura, Y., Kaneko, T., Awai, K., Amagai, M., Kuwata, C., Tsugane, T., Masuda, T., Shimada, H., Takamiya, K., Ohta, H. and Tabata, S. (2001). Monitoring of methyl jasmonate-responsive genes in *Arabidopsis* by cDNA microarray: Self-activation of jasmonic acid biosynthesis and crosstalk with other phytohormone signaling pathways. DNA Res. **8**. 153-161.

Schaller, F. (2001). Enzymes of the biosynthesis of octadecanoid-derived signaling molecules. J. Exp. Bot. **52**, 11-23.

Schaller, F., Biesgen, C., Mussig, C., Altmann, T. Weiler, E.W. (2000). 12-oxophytodienoate reductase 3 (OPR3) is the isoenzyme involved in jasmonate biosynthesis. Planta **210**, 979-984.

Schaller, F., Hennig, P. and Weiler, E.W. (1998). 12-Oxophytodienoate-10,11 reductase: Occurrence of two isoenzymes of different specificity against stereoisomers of 12-oxophyto- dienoic acid. Plant Physiol. **118**, 1345-1351.

Schaller, F. and Weiler, E.W. (1997a). Enzymes of octadecanoid biosynthesis in plants: 12-Oxo-phytodienoate 10,11-reductase. Eur. J. Biochem. **245**, 294-299.

Schaller, F. and Weiler, E.W. (1997b). Molecular cloning and characterization of 12-oxophytodienoate reductase, an enzyme of the octadecanoid-signaling pathway from *Arabidopsis thaliana*: Structural and functional relationship to yeast old yellow enzyme. J. Biol. Chem. **272**, 28066-28072.

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C. and Manners, J.M. (2000). Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. Proc Natl. Acad. Sci USA.**97**, 11655-11660.

Schindler, U., Menkens, A.E., Beckmann, H., Ecker, J.R. and Cashmore, A.R. (1992). Heterodimerization between light-regulated and ubiquitously expressed Arabidopsis GBF bZIP proteins. EMBO J. **11**, 1261-1273.

Schmidt, J., Kramell, R. and Schneider, G. (1995). Liquid chromatography electrospray tandem mass spectrometry of amino acid conjugates of jasmonic acid under positive and negative ionization. European Mass Spectrometry **1**, 573-581.

Schraudner, M., Moeder, W., Wiese, C., Van Camp, W., Inze, D., Langebartles, C., Sandermann, H. Jr. (1998). Ozone-induced oxidative burst in the ozone biomonitor plant, tobacco Bel W3. Plant J. **16**, 235-245.

Sembdner, G. and Parthier, B. (1993). The biochemistry and the physiological and molecular actions of jasmonates. Ann. Rev. Plant Physiol. Plant Mol. Biol. **44**, 569-589.

Seo, H.S., Song, J.T., Cheong, J.-J., Lee, Y.-H., Lee, Y.-W., Hwang, I., Lee, J. S. and Choi, Y. D. (2001). Jasmonic acid carboxyl methyltransferase: A key enzyme for jasmonate-regulated plant responses. Proc. Natl. Acad. Sci. USA **98**, 4788-4793.

Seo, S., Okamoto, M., Settee, H., Ishizuka, K., Sano, H. and Ohashi, Y. (1995). Tobacco MAP kinase: A possible mediator in wound signal transduction pathways. Science **270**, 1988-1992.

Skowyra, D., Craig, K.L., Tyers, M., Elledge, S.J. and Harper, J.W. (1997). F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex. Cell **91**, 209-219.

Shah, J., Kachroo, P., Nandi, A. and Klessig, D.F. (2001). A recessive mutation in the *Arabidopsis SSI2* gene confers SA- and *NPR1*-independent expression of *PR* genes and resistance against bacterial and oomycete pathogens. Plant J. **25**, 563-574.

Song, W.C., Funk, C.D. and Brash A.R. (1993). 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.

Staswick, P. E., Su, W. and Howell, S. H. (1992). Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. Proc. Nat. Acad. Sci. USA **89**, 6837-6840.

Staswick, P.E., Yuen, G.Y. and Lehman, C.C. (1998). Jasmonate signaling mutants of *Arabidopsis* are susceptible to the soil fungus *Pythium irregulare*. Plant J. **15**, 747-754.

Stelmach, B.A., Müller, A., Hennig, P., Gebhardt, S., Schubert-Zsilavecz, M. and Weiler, E.W. (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**.**

Stelmach, B.A., Muller, A., Hennig, P., Laudert, D., Andert, L. and Weiler, E.W. (1998). Quantitation of the octadecanoid 12-oxo-phytodienoic acid, a signaling compound in plant mechanotransduction. Phytochemistry. **47**, 539-546.

Stintzi, A. and Browse, J. (2000). The *Arabidopsis* male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. Proc. Natl. Acad. Sci. USA **97**, 10625-10630.

Stintzi, A., Weber, H., Reymond, P., Browse, J. and Farmer, E.E. (2001). Plant defense in the absence of jasmonic acid: the role of cyclopentenones. Proc. Natl. Acad. Sci. USA **98**, 12837-12842.

Thomma, B.P.H.J., Eggermont, K., Penninckx, I.A.M.A., Mauch-Mani, B., Vogelsang, R., Cammue, B.P.A. and Broekaert, W.F. (1998). Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. Proc. Natl. Acad. Sci. USA **95**, 15107-15111.

Thompson, J.E., Froese, C.D., Madey, E., Smith, M.D. and Hong, Y. (1998). Lipid metabolism during plant senescence. Prog. Lipid Res. **37**, 119-141.

Utsugi, S., Sakamoto, W., Ogura, Y., Murata, M. and Motoyoshi, F. (1996). Isolation and characterization of cDNA clones corresponding to the genes expressed preferentially in floral organs of *Arabidopsis thaliana*. Plant Mol. Biol. **32**, 759-765.

Vick, B.A. (1993). Oxygenated fatty acids of the lipoxygenase pathway. In Lipid Metabolism in Plants, T. S. Moore Jr., ed (Boca Raton: CRC Press), pp. 167-191.

Vijayan, P., Shockey, J., Levesque, C.A., Cook, R.J. and Browse J. (1998). A role for jasmonate in pathogen defense of *Arabidopsis*. Proc. Natl. Acad. .Sci. USA. **95**, 7209-7214.

Vollenweider, S., Weber, H., Stolz, S., Chetelat, A. and Farmer, E.E. (2000). Fatty acid ketodienes and fatty acid ketotrienes: Michael addition acceptors that accumulate in wounded and diseased *Arabidopsis* leaves. Plant J. **24**, 467-476.

Wang, C., Zien, C., Afitlhile, M., Welti, R., Hildebrand, D.F. and Wang, X. (2000). Involvement of phospholipase D in wound-induced accumulation of jasmonic acid in *Arabidopsis*. Plant Cell, **12**, 2237-2246.

Wang, C.-L. and Oliver, D.J. (1966). Cloning of the cDNA and genomic clones for glutathione synthetase from Arabidopsis thaliana and complementation of a gsh2 mutant in fission yeast.
Plant Mol. Biol. **31,** 1093-1104.

Wang, X. (1999). The role of phospholipase D in signaling cascades. Plant Physiol. **120**, 645-651.

Weber, H., Vick, B.A. and Farmer, E.E. (1997). Dinor-oxo-phytodienoic acid: a new hexadecanoid signal in the jasmonate. Proc. Natl. Acad. Sci. USA **94**, 10473-10478.

van Wees, S.C., de Swart, E.A., van Pelt, J.A., van Loon, L.C. and Pieterse, C.M.(2000). Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **97**, 8711-8716.

Xiang, C. and Oliver, D.J. (1998). Glutathione metabolic genes coordinately respond to heavy metals and jasmonic acid in *Arabidopsis*. Plant Cell **10**, 1539-1550.

Xie, D.X., Feys, B.F., James, S., Nieto-Rostro, M. and Turner, J.G. (1998). *COI1*: an *Arabidopsis* gene required for jasmonate-regulated defense and fertility. Science **280**, 1091-1094.

Xu, Y., Chang, P.F.L., Liu, D., Narasimhan, M.L., Raghothama, K.G., Hasegawa, P.M. and Bressan, R.A. (1994). Plant defense genes are synergistically induced by ethylene and methyl jasmonate. *Plant Cell* **6,** 1077-1085.

Xu, L., Liu, F., Wang, Z., Peng, W., Huang, R., Huang, D. and Xie, D. (2001). An *Arabidopsis* mutant *cex1* exhibits constant accumulation of jasmonate-regulated AtVSP, Thi2.1 and PDF1.2. FEBS Lett. **494**, 161-164.

Yokoyama, M., Yamaguchi, S., Inomata, S., Komatsu, K., Yoshida, S., Iida, T., Yokokawa, Y., Yamaguchi, M., Kaihara, S. and Takimoto, A. (2000). Stress-induced factor involved in flower formation of *Lemna* is an a-ketol derivative of linolenic acid. Plant Cell Physiol. **41**, 110-113.

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

Ziegler, J., Stenzel, I., Hause, B., Maucher, H., Hamberg, M., Grimm, R., Ganal, M. and Wasternack, C. (2000). Molecular cloning of allene oxide cyclase. The enzyme establishing the stereochemistry of octadecanoids and jasmonates. J. Biol. Chem. **275**, 19132-19138.