Nonenzymatic Oxidation of Trienoic Fatty Acids Contributes to Reactive Oxygen Species Management in Arabidopsis*

Received for publication, September 15, 2008, and in revised form, November 6, 2008

Laurent Mène-Saffrané, Lucie Dubugnon, Aurore Chételat, Stéphanie Stolz, Caroline Gouhier-Darimont, and Edward E. Farmer

From the Plant Molecular Biology Department, University of Lausanne, Biophore, 1015 Lausanne, Switzerland

In higher plants such as Arabidopsis thaliana, omega-3 trienoic fatty acids (TFAs), represented mainly by α-linolenic acid, serve as precursors of jasmonic acid (JA), a potent lipid signal molecule essential for defense. The JA-independent roles of TFAs were investigated by comparing the TFA- and JA-deficient fatty acid desaturase triple mutant (fad3-2 fad7-2 fad8 (fad3 fad7 fad8)) with the aos (allene oxide synthase) mutant that contains TFAs but is JA-deficient. When challenged with the fungus Botrytis, resistance of the fad3 fad7 fad8 mutant was reduced when compared with the aos mutant, suggesting that TFAs play a role in cell survival independently of being the precursors of JA. An independent genetic approach using the lesion mimic mutant accelerated cell death2 (acd2-2) confirmed the importance of TFAs in containing lesion spread, which was increased in the lines in which the fad3 fad7 fad8 and acd2-2 mutations were combined when compared with the aos acd2-2 lines. Malondialdehyde, found to result from oxidative TFA fragmentation during lesion formation, was measured by gas chromatography-mass spectrometry. Its levels correlated with the survival of the tissue. Furthermore, plants lacking TFAs overproduced salicylic acid (SA), hydrogen peroxide, and transcripts encoding several SA-regulated and SA biosynthetic proteins. The data suggest a physiological role for TFAs as sinks for reactive oxygen species.

The trienoic fatty acid (TFA), \( \alpha \)-linolenic acid (ALA), is the most abundant fatty acid in the aerial tissues of most plants and is among the most abundant fatty acids on earth. ALA can reach up to 54 mol % of leaf fatty acids in the plant Arabidopsis thaliana (1) and is particularly prevalent in the membranes of chloroplasts (2, 3) and mitochondria (3, 4). Finding TFAs in these cellular locations is of considerable interest given the fact that these organelles are sites of production of reactive oxygen species (ROS) and that ALA is particularly sensitive to oxidation in the presence of these reactive species (5). Given its abundance in plants, it is remarkable that TFAs are not essential for photosynthesis and vegetative growth of Arabidopsis in the laboratory (6). Instead, TFAs in leaves have roles in environmental stress and in defense responses. At low temperatures, TFA depletion in Arabidopsis both compromises recovery from photoinhibition (7) and also impairs the process of chloroplast maintenance (8). In bacterial pathogenesis, the translocation of ALA from the chloroplast to extrachloroplastic membranes may help activate NADPH oxidase, an enzyme responsible for the production of reactive oxygen species necessary for full resistance to avirulent pathogens (9). However, the best characterized role of TFAs is as an essential precursor in jasmonic acid (JA) biosynthesis (10).

JA is the precursor of potent signals that regulate the expression of many defense genes in plants. For example, ~30% of Arabidopsis genes differentially expressed in response to infection with the fungus Botrytis cinerea depend on a functional JA signal pathway for their correct expression (11). Moreover, JA mutants lacking the ability to accumulate or to perceive JA present an increased susceptibility to Phythium mastophorum (12) and to Alternaria brassicicola and Botrytis cinerea (13). In keeping with this, jasmonates often accumulate to high levels in diseased Arabidopsis where oxylipin generation, particularly during lesion formation, can be extensive (14). Independently of JA, other products of lipid oxidation may also play roles in gene expression. Among these are biologically active oxidized lipids and lipid fragments including reactive electrophile species, many of which are derived in vivo from TFAs (15). These range from small highly ubiquitous compounds such as malondialdehyde (MDA (16, 17)) to families of hydroxy-fatty acids and phytosteranes (14). The potential biological activities of TFAs and TFA-derived compounds needs further investigation, but their activities could, potentially, be masked by the powerful effects of JA.

To investigate whether TFAs have an intrinsic role independent of being the precursors of JA, we compared the response of the fad3-2 fad7-2 fad8 (fad3 fad7 fad8) and the aos mutant challenged with pathogens. In separate experiments, the progress of lesion formation was followed in the lesion mimic mutant acd2-2 in the presence and absence of TFAs. The mutant fad3 fad7 fad8 lacks both TFAs and JA, whereas the aos mutant is used as a control because it lacks only JA. The position of these mutants in TFA and JA synthesis is illustrated in...
Fig. 1A. The results obtained in two different systems employing these mutants show that TFAs but not JA were essential for full plant protection. MDA measurements revealed that TFAs contribute significantly to ROS-related homeostasis. Salicylic acid (SA) measurements and gene expression studies also revealed differences between the fad3 fad7 fad8 mutant (6), the aos mutant (18), and the acd2-2 mutant (19) in the Col-0 background. The aos mutant contains the gl1 mutation (GLABROUS1) that was removed by backcrossing to Col-0. Homozygous GL1/GL1 aos plants were identified in the F2 population by PCR as described previously (18) as well as for their male sterile phenotype and the presence of trichomes. Sterility was rescued by spraying methyl jasmonate (MeJA) (0.01% in 0.01% Tween 20) at 2-day intervals during flowering. Plants were grown at 90 μmol m−2 s−1 and 22 °C under a 10-h light/14-h dark photoperiod. Seeds were stratified for 3–5 days at 4 °C. B. cinerea was grown on potato dextrose agar (3.9% w/v; BD Biosciences) to obtain conidiospores. Inoculations were performed with 104 spores/ml in 0.6% potato dextrose broth. Four droplets (6 μl each) were applied per plant (4-week-old) to separate, fully expanded leaves. Plants were placed in saturating humidity and constant light (100 microeinsteins m−2 s−1) at 22 °C. Inoculations with Hyaloperonospora arabidopsidis (formerly Peronospora parasitica (Noco2)) were performed as described in Ref. 20.

Small Molecule Measurement and DAB Staining—Chlorophyll was extracted from lyophilized tissues without grinding using 15–20 ml of dimethyl sulfoxide in the dark at 65 °C overnight. Chlorophyll content was determined spectrophotometrically at 645 and 663 nm (21). JA was determined using 18O-JA as an internal standard (14). For MDA measurements, a quantitative gas chromatography/mass spectrometry test was based on the formation of a specific pentfluorophenylhydrazine:MDA adduct was used together with [3H2]malondialdehyde as an internal standard (22). SA was measured according to Ref. 23. Free SA was extracted from 130 mg of powder, whereas total SA was extracted from 70 mg of powder. The flow rate of the mobile phase was 0.9 ml/min. 3,3′-Diaminobenzidine (DAB) staining, an indicator of hydrogen peroxide levels, was performed for 15 h at 22 °C according to Ref. 24.

Double Mutant and Quadruple Mutants—The aos GL1/GL1 mutant was fertilized with acd2-2 pollen. The homozygous aos plants were isolated from the F2 population by PCR analysis in a reaction carried out with an annealing temperature of 55 °C using three primers: F1, 5′-GGGAGCGATTGAGAAAATGG-3′, and R1, 5′-CGCGAGAAATTAACGGAGC-3′, amplify a fragment of 442 bp from the WT allele, whereas the F2, 5′-CGG-GCCAACCTTTGTTGTTGATGCT-3′, in combination with R1 amplifies a fragment of 268 bp in the T-DNA interrupted AOS gene. Homozygous aos acd2-2 was isolated using the strategy outlined above for the fad3 fad7 fad8 acd2-2 mutant. The fad3 fad7 fad8 mutant (6) was fertilized with acd2-2 pollen. All initial attempts to isolate fad3 fad7 fad8 acd2-2 quadruple mutants from ~5,500 F2 plants failed because putative quadruple mutants died under greenhouse conditions and under long day conditions (more than 11 h) in growth rooms. Consequently, F2 male sterile plants heterozygous for ACD2 were selected to isolate F3 homozygous quadruple mutants in short day conditions (9-h days at relatively low light). Under these permissive conditions, the quadruple mutants produced seed, although plants were severely damaged. The absence of TFAs in the male sterile F2 plants was confirmed by chromatographic analysis (25). Homozygous acd2-2 mutants were identified at the F3 generation by cleaved amplified polymorphism sequence analysis. For ACD2/acd2-2 allele differentiation, a 387-bp-long fragment was amplified with the forward primer 5′-GTGAAACAGCTTGGTCCGATGATGCT-3′ and the reverse primer 5′-CCGACAAAGAGAGAAGGAGAC-3′ in a PCR reaction carried out with an annealing temperature of 53 °C. Restriction of acd2-2 alleles with TaSI endonuclease (Fermentas) generated two fragments (299 and 84 bp), whereas ACD2 was not digested. The quadruple mutant was maintained by spraying developing inflorescences daily with MeJA (0.01% v/v) under 10 h of light at 100 microeinsteins m−2 s−1. Manual pollination of flowers was necessary to obtain sufficient seed. Under all conditions examined, the quadruple mutants showed more severe lesion formation than the doubles, but the exact timing and extent of lesion formation depends on soil humidity, light, and nutrient conditions and is thus variable.

Microarray Experiments—To compare transcript levels between the fad3 fad7 fad8 and the aos mutant, gene expression levels in each mutant were determined using Col-0 as reference in triplicate biologically independent experiments. Complete Arabidopsis Transcriptome MicroArrays (CATMA (26)) were used. Genes were considered up- or down-regulated in the mutants versus Col-0 when the absolute value of the expression ratio (log2) mutant/WT > 1 and p < 0.05. Genes were considered differentially expressed between the fad3 fad7 fad8 and the aos mutant when p < 0.05. Total RNA was extracted from 6-week-old plants grown in 10-h light (100 microeinsteins m−2 s−1) at 22 °C. Protocols for RNA extraction, labeling, hybridization, scanning, and statistics have been previously described (27) and are also available with the microarray data through ArrayExpress under accession numbers E-ATMX-36 (for the aos mutant) and E-ATMX-37 (for the fad3 fad7 fad8 mutant).

RESULTS

Full Survival of Infection Requires TFAs but Not JA—To investigate a potential JA-independent role of TFAs in the response of Arabidopsis to B. cinerea, the phenotype of the TFA- and JA-deficient mutant, fad3 fad7 fad8 (6), was compared with the aos mutant (allene oxide synthase) that lacks the ability to synthesize JA but which contains TFAs (Fig. 1A) (14, 18). No marked differences in lesion formation were observed between the two mutants 2 days after inoculation (supplemental Fig. S1A). However, when disease was allowed to develop over longer periods (9 days), a strong difference was observed between the response of the fad3 fad7 fad8 and the aos mutant...
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**A**

Linoleic acid

\[ \text{fad3 fad7 fad8} \]

α-Linolenic acid

MDA

Jasmonic acid

**B**

*Botrytis cinerea*

**C**

Leaf survival (%)

| Genotype | 0% | 25% | 50% | 75% | 100% |
|----------|-----|-----|-----|-----|------|
| Col-0    |     |     |     |     | 100  |
| fad3 fad7 fad8 aos | | | | | 100 |

**FIGURE 1.** Oxidation of ALA and survival of *Arabidopsis* mutants inoculated with pathogens. **A**, enzymatic oxidation of ALA leads to JA synthesis, whereas its nonenzymatic oxidation by ROS generates MDA. The fad3 fad7 fad8 mutant lacks TFAs and JA and contains only 25% of WT MDA. The aos mutant is JA-deficient but contains TFAs. **B**, phenotypes of the wild type (Col-0) and two mutants challenged with *B. cinerea*. **C**, leaf survival (bars, average ± S.E.; n = 6) and total chlorophyll (open bars, average ± S.E.; n = 5) 9 days after inoculation. * and **, p < 0.05 and 0.01, respectively by Student’s test relative to the aos mutant. DW = dry weight.

At this time point, the youngest central rosette leaves of the fad3 fad7 fad8 mutant died, whereas those of both WT and the aos mutant remained alive. Although 82.9% of WT leaves survived inoculation, fewer (51.2%) leaves in the JA-deficient mutant aos survived. However, the effect of the fad3 fad7 fad8 mutations lacking both JA and TFAs was far greater, and only 3.8% of these leaves survived (Fig. 1C). Furthermore, ~50% of the aos plants survived and produced seed, whereas all the fad3 fad7 fad8 plants died before producing any seeds. In agreement with this, chlorophyll levels were lower in *Botrytis*-infected fad3 fad7 fad8 plants than in aos plants (Fig. 1C). Furthermore, treatment with exogenous MeJA strongly protected aos plants from *Botrytis* infections but only partially protected the fad3 fad7 fad8 plants (supplemental Fig. S2). This increased sensitivity of the TFA-deficient mutant was also observed in response to the biotrophic oomycete *H. arabidopsidis* as judged by the number of diseased leaves and by the chlorophyll content (supplemental Fig. S1B). The results showed that properties of TFAs independent of jasmonate production are required for resistance of *Arabidopsis* to *B. cinerea* and *H. arabidopsidis*.

Recapitulation of the Cell Survival Phenotype in a Genetic Model—To investigate whether the presence of TFA could affect cell survival in the absence of a pathogen, a lesion mimic mutant was selected for further study. The accelerated cell death 2-2 mutant (acd2-2) displays spontaneous disease-like lesions and was chosen because the onset of lesion formation is highly predictable and, under appropriate conditions, relatively slow (supplemental Fig. S3). This latter point was considered to be critical given that the effect of TFAs in promoting plant survival of *Botrytis* infection was observed only over relatively long time scales. ROS production is well documented in acd2-2 (28), and we found that TFA oxidation was also accelerated in acd2-2 undergoing lesion formation where both MDA and JA accumulate (supplemental Fig. S3). To test the consequence of TFA depletion on lesion formation, the acd2-2 mutant was crossed into the fad3 fad7 fad8 mutant background. Lesion formation in three different quadruple mutant lines was accelerated with respect to acd2-2 (Fig. 2A) with lesions developing on most of the rosette. Lesions spread from expanded leaves into the growing centers of the quadruple mutants, resulting in their death. The presence of TFAs in acd2-2 strongly impedes centripetal lesion progression into young tissues. Quadruple mutant seeds grown under axenic conditions also displayed severe tissue damage under short day conditions also displayed severe tissue damage under short day conditions.
conditions (not shown), ruling out opportunistic pathogens as a cause of lesion formation in the quadruple mutant.

JA has been reported to inhibit lesion formation as a consequence of ozone treatment (29, 30). To test whether the selective removal of jasmonate from the acd2-2 mutant led to more rapid cell damage, double mutants were generated from the aos and the acd2-2 mutants and compared with the fad3 fad7 fad8 acd2-2 mutants. The aos acd2-2 plants displayed more severe lesions than the acd2-2 plants, but tissue death was less rapid and much less extensive than in the fad3 fad7 fad8 acd2-2 plants (Fig. 2A). Chlorophyll measurements confirmed these observations and showed greater losses in the quadruple mutants when compared with the double mutants (supplemental Fig. S4). Furthermore, lesions in the aos acd2-2 double mutants did not spread efficiently to the growing center of the plants, most of which survived. Plant survival was monitored over time and showed a first difference between the 3rd and the 4th week when several fad3 fad7 fad8 acd2-2 plants died, whereas all the aos acd2-2 plants survived (Fig. 2B). This increased mortality was also observed after 4, 5, and 6 weeks, times at which less than 40% of fad3 fad7 fad8 acd2-2 plants survived, whereas ~80% of the aos acd2-2 plants remained alive.

Altered ROS Homeostasis in the TFA-deficient Mutant—Resistance to two pathogens or to spreading lesion formation in the acd2-2 mutant requires TFAs independent of JA synthesis. In both cases, long duration oxidative stresses are involved; therefore, the essential role of TFAs may be associated with their availability for oxidation. The oxidation of TFAs by ROS leads to the generation of many lipid oxidation products including MDA (Fig. 1A) and to their subsequent turnover (17). To estimate the extent of TFA oxidation in different genetic backgrounds containing the acd2-2 mutation, MDA levels were determined. In the absence of lesions, Col-0 and the acd2-2 mutant have similar levels of MDA, whereas the fad3 fad7 fad8 mutant contains only 20% of WT level (Fig. 3A, white bars). In the aos mutant, MDA levels correspond to 65% of Col-0 level, showing that ~35% of the MDA pool in a healthy tissue is maintained in an AOS-dependent manner. In the different lines of the aos acd2-2 and the fad3 fad7 fad8 acd2-2 mutants, levels of MDA were similar to the aos and the fad3 fad7 fad8 parents, respectively. However, when visible lesions appear, the MDA levels increase in all the genotypes analyzed (Fig. 4A, black bars). The level of MDA in acd2-2 leaves is increased by 1.68-fold to reach 97.3 nmol g⁻¹ dry weight (100%). In the aos acd2-2 mutant lines D1 and D3, MDA levels are similar to the acd2-2 parent (100 and 97% respectively), whereas the line D2 presents a higher amount of MDA (134%). In the fad3 fad7 fad8 acd2-2 mutant lines Q1, Q2, and Q3, the appearance of lesions also correlates with an increase of MDA of 2.3-, 2.0-, and 1.7-fold, respectively; however, these MDA levels were only 17, 14, and 13% of those in acd2-2 leaves with lesions. Because MDA production is the result of ROS action on TFAs, these data demonstrate that ROS associated with the acd2-2 mutation are consumed in the acd2-2 and the aos acd2-2 plants through TFA oxidation. Evidence consistent with altered hydrogen peroxide homeostasis in TFA-deficient plants came from DAB staining, which was more intense in the plant lines carrying the fad3 fad7 fad8 mutations (Fig. 3B).

Altered Transcript and SA Levels in the Absence of TFAs—In control conditions, the wild type and the aos mutant accumulates much more MDA than the fad3 fad7 fad8 mutant (Fig. 3A), indicating that TFA oxidation is a natural process that takes place constantly in healthy resting plants and must be linked to basal metabolism. To test the consequences of this intrinsic property of TFAs for gene expression, transcript levels were compared between the aos and the fad3 fad7 fad8 mutants using printed microarrays displaying nearly 20,000 genes. For this, gene expression levels in each mutant were determined by comparing either wild type and the aos acd2-2 mutant (see “Experimental Procedures”). From these data, transcript level ratios were compared between the aos and the fad3 fad7 fad8 mutant. These data are available on ArrayExpress. This analysis revealed that 1,610 genes were differentially expressed in the fad3 fad7 fad8 and the aos mutant (Fig. 4A). A sublist of genes up- or down-regulated in the fad3 fad7 fad8 mutant but unchanged in the aos mutant was established (supplemental Table S1), revealing 68 transcripts up-regulated in the fad3 fad7 fad8 mutant but not in the aos mutant. It is noticeable that the function of several of these genes is related to plant defense because this list encompasses the genes encoding the pathogenesis-related proteins PR-1 and PR-5, the avirulence-induced gene AIG1, the gene encoding the phytoalexin-deficient 4 protein PAD4, and two putative disease resistance proteins. Moreover, this list also includes two genes involved in SA biosynthesis ICS1 and SID1 as well as the gene encoding NPR1/NIM1-interacting protein.

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**FIGURE 3. Lipid oxidation and evidence of oxidative stress in leaves.** A, MDA levels were quantified with a specific assay in leaves without [ ] or with lesions [ ] (average ± S.E.; n = 6–10). Genotypes are indicated at the right of each bar. Wild type is Col-0; Q1, Q2, and Q3 and D1, D2, and D3 are three different lines of the fad3 fad7 fad8 acd2-2 and the aos acd2-2 mutant, respectively. ***, p < 0.01 by Student’s t test relative to the aos acd2-2. Note that WT, the fad mutant, and the aos mutant at the top of the figure do not produce lesions under laboratory growth conditions. B, in situ DAB staining for hydrogen peroxide in 5-week-old plants.
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FIGURE 4. SA levels and gene expression in the wild type (Col-0), the fad3 fad7 fad8 mutant, and the aos mutant. A, expression ratios (log2) of the fad3 fad7 fad8 mutant are plotted against expression ratios (log2) of the aos mutant. Only genes differentially expressed between the two mutants (p < 0.05) are represented on the plot. Pink and green spots symbolize genes up- or down-regulated relative to the WT in at least one of the mutants (absolute value ratio > 1; p < 0.05). Blue spots represent genes not up- or down-regulated relative to the WT (both absolute value ratio < 1 or one absolute value ratio > 1 with the associated p > 0.05). Green spots symbolize genes presented in panel B. B, sublist of genes up-regulated only in the fad3 fad7 fad8 mutant and the respective expression ratio (log2) in both mutants. C, free SA (□) and total SA (■) in 6-week-old plants (average ± S.E., n = 5 or 6). EW = fresh weight.

NIMIN-2 (Fig. 4B), suggesting that activity of the SA pathway was enhanced in the TFA-deficient but not in the aos mutant. This was confirmed by SA quantification. The TFA-deficient mutant contained 2.1- and 15.2-fold more free and total SA, respectively, than the aos mutant (Fig. 4C).

DISCUSSION

ALA and related omega-3 fatty acids are particularly sensitive to oxidation by ROS (5) and are abundant in the membranes of organelles known to produce ROS. This apparent paradox could suggest that oxidation of TFAs by ROS could have undiscovered physiological functions. However, more is known about TFAs as being the precursors of JA. Indeed, the first Arabidopsis mutant lacking TFAs, the fad3 fad7 fad8 mutant, was male sterile (6), susceptible to pathogens and insects (12, 31), and susceptible to environmental stress (7, 8, 32). In most of these cases, the application of exogenous JA or MeJA was sufficient to restore plant fertility or to protect significantly from stress, therefore confirming the critical function of TFAs as the precursors of JA.

To define whether TFAs have roles independent of being the precursors of JA, we compared the response of the fad3 fad7 fad8 mutant and the aos mutant challenged with either the fungus Botrytis or the oomycete Hyaloperonospora. Both TFAs and JA are depleted in the fad3 fad7 fad8 mutant, whereas the aos mutant lacks JA but contains TFAs. Our results showed that the fad3 fad7 fad8 mutant is more susceptible to Botrytis than the aos mutant (Fig. 1). Similar results were obtained with a pathogenic oomycete. These data demonstrated that TFAs are not only the precursors of JA but have additional roles in the defense of Arabidopsis against pathogens. We also analyzed the role of TFAs in the absence of pathogens by using the lesion mimic mutant acd2-2 (19, 28). Again we observed a more protective role of TFAs than of products of the AOS enzyme in slowing lesion spread (Fig. 2). These results may be informative for understanding why plants lacking TFAs can be more vulnerable to pathogens than WT plants. Reduced leaf survival in Botrytis infections may be due to the deregulated cell death seen in the quadruple fad3 fad7 fad8 aos acd2-2 mutant.

Finding a protective effect of TFAs in different systems demonstrates the importance of these omega-3 fatty acids in tissue health. Because TFAs are particularly sensitive to nonenzymatic oxidation mediated by ROS, we analyzed one of the products of these reactions: MDA. MDA production is strongly stimulated in the aos acd2-2 and in the acd2-2 plants during lesion formation, showing that a part of ROS produced in response to the acd2-2 mutation is consumed through TFA oxidation (Fig. 3 and supplemental Fig. S3). Moreover, the comparison of MDA levels between wild type and fad3 fad7 fad8 mutants in control conditions showed that MDA is also constantly produced in healthy control plants (Fig. 3 (17)). These data suggest that TFA-mediated ROS consumption resulting in the nonenzymatic oxidation of polyunsaturated fatty acids is a common and constant biological process. We propose the hypothesis that this may be a mechanism by which cells control ROS levels. Moreover, the pressure on the cell to manage ROS production increases over the relatively long duration of disease development or environmental stress, and our experiments suggest that it is in such conditions that TFA oxidation is vital as a way to remove ROS. Importantly, treatments with exogenous compounds known to generate ROS (e.g. herbicides such as Diquat) did not differentially affect the aos and the fad3 fad7 fad8 mutants (not shown). This is perhaps not surprising given that the protective role of TFAs may function best when ROS is produced chronically, such as in the
acld2 mutant. Herbicide treatment is abrupt and does not fully mimic the chronic ROS generation produced through genetic interventions.

Consistent with the hypothesis that TFAs help to protect cells by absorbing ROS was the fact that the fad3 fad7 fad8 plants stained more heavily for hydrogen peroxide than did the aos or the wild-type plants (Fig. 3B). This suggests that the TFAs present in aos and in the wild-type plants are able to consume ROS. This also indicates that the fad3 fad7 fad8 mutant is under constant oxidative stress and that this might lead to effects on gene expression. These observations were supported by the fact that these plants contained elevated levels of SA. In plants, SA is a regulator of oxidative stress-related gene expression, and its accumulation is known to be intimately associated with ROS homeostasis, for example, during pathogenesis (33). Finding these biochemical features of the fad3 fad7 fad8 plants prompted us to investigate gene expression. The outcome of these experiments revealed that among the transcripts up-regulated in the fad3 fad7 fad8 mutant but not in the aos mutant were genes encoding pathogenesis-related (PR)-proteins and salicylate biosynthetic proteins. Clearly, the expression of these and other differentially regulated genes is normally dampened by TFAs independently of JA (Fig. 4).

From other work with the ssi2 mutant, a correlation between desaturation of plastidic fatty acids and PR proteins/SA pathway up-regulation has been attributed to the reduction of 18:1 levels and the concomitant increase in the levels of 18:0 (34–36). However, a similar mechanism would not explain our results because the fad3 fad7 fad8 mutant is deficient in TFAs and has slightly increased 18:1 levels, whereas the ssi2 mutant has normal levels of TFAs and a strong reduction of 18:1 (34). Our results support a role of TFAs in ROS metabolism or redox homeostasis because many PR genes/SA biosynthetic genes are redox-sensitive genes induced in response to biotic or abiotic stresses (37–39). Moreover, it is interesting to note that stress-related genes up-regulated in the fad3 fad7 fad8 mutant are also up-regulated in the ascorbate-deficient mutant vtc1 (40). Finding these genes up-regulated in the TFA-deficient fad3 fad7 fad8 mutant corroborates the results of DAB staining for hydrogen peroxide and SA measurement and indicates that the mutant is under oxidative/redox stress that contributed to increased vulnerability to pathogens.

We conclude that having high concentrations of omega-3 triunsaturated fatty acids in chloroplasts and mitochondria and elsewhere would make sense if these compounds, via the production of small oxygenated compounds such as MDA, absorb a part of the ROS generated in these organelles. This proposed route to the elimination of reactive oxygen species would be hemimetabolic because it is initiated by nonenzymatic fatty acid fragmentation. Because the TFAs are already present, this mechanism would present the advantage of immediately consuming ROS, unlike the regulated activation of genes encoding ROS-catabolizing enzymes. An understanding of the metabolism of fatty acid oxidation products including MDA will be necessary to further investigate this proposed route of ROS elimination.

**Acknowledgments**—We thank A. Gfella for aos in a GL1 background, C. Funkhauser, H. Weber, and S. Hörtensteiner for helpful discussion, and N. Geldner, Y. Poirier, and U. Paszkowski for valuable comments on the manuscript. D. DellaPenna generously provided support for SA analyses, J. E. Parker, M. Straus, and B. Mauch-Mani gave us Hyaloperonospora and helpful advice, and P. Reymond provided technical support for microarray experiments.

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Omega-3 Fatty Acids and ROS