FEBS Letters 586 (2012) 2483–2487

journal homepage: www.FEBSLetters.org

# Oxo-phytodienoic acid (OPDA) is formed on fatty acids esterified to galactolipids after tissue disruption in *Arabidopsis thaliana*

Anders K. Nilsson, Per Fahlberg, Mats Ellerström *, Mats X. Andersson *

*Department of Biological and Environmental Sciences, University of Gothenburg, Box 461, SE-405 30 Gothenburg, Sweden*

ARTICLE INFO

*Article history:*
Received 21 April 2012
Revised 3 June 2012
Accepted 7 June 2012
Available online 19 June 2012

Edited by Ulf-Ingo Flügge

*Keywords:*
Jasmonate
Oxo-phytodienoic acid
Galactolipid
Plastid

ABSTRACT

**Biotic and abiotic stress induces the formation of galactolipids esterified with the phytohormones 12-oxo-phytodienoic acid (OPDA) and dinor-oxo-phytodienoic acid (dnOPDA) in *Arabidopsis thaliana*. The biosynthetic pathways of free (dn)OPDA is well described, but it is unclear how they are incorporated into galactolipids. We herein show that (dn)OPDA containing lipids are formed rapidly after disruption of cellular integrity in leaf tissue. Five minutes after freeze-thawing, 60–70% of the trienoic acids esterified to chloroplast galactolipids are converted to (dn)OPDA. Stable isotope labeling with $^{18}$O-water provides strong evidence for that the fatty acids remain attached to galactolipids during the enzymatic conversion to (dn)OPDA.**
**© 2012 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.**

## 1. Introduction

The chloroplasts membrane lipids mono- (MGDG) and digalactosyl diacylglycerol (DGDG) are rich in trienoic fatty acids. Apart from their obvious role in maintaining membrane physiology, these fatty acids function as precursors for a large family of signaling compounds collectively known as oxylipins [1]. Of these, the best characterized are the jasmonates [2,3]. Jasmonates are formed by the consecutive action of 13-lipoxygenase, allene oxide-synthase and cyclase on linolenic (18:3) or hexadecatrienoic (16:3) acid in the plastid stroma [4]. The immediate product of this pathway is oxo-phytodienoic acid (OPDA) or dinor-OPDA (dnOPDA), both of which have signaling properties by themselves [5–9]. These are converted to the phytohormone jasmonic acid in the peroxisome and then activated by conjugation to isoleucine [2,3]. The pathway as a whole is thought to work on free fatty acids liberated from glycerolipids producing (dn)OPDA in the plastid [1,3,4].

*Arabidopsis thaliana* contains OPDA and dnOPDA esterified to the glycerol backbone and/or the galactose headgroup of plastid membrane galactolipids [8,10–18]. These compounds are sometimes referred to as Arabidopsides and were found to accumulate after elicitation by bacterial pathogenic effectors [14,15,17,19], wounding [15,17,18,20], senescence [16] and cold treatment [17]. Arabidopsides have been linked to insect defense [21] and might act as antibacterial [14] and antifungal compounds [15]. It still remains elusive if bound (dn)OPDA is synthesized through a free fatty acid intermediate or synthesized while the fatty acids remain attached to the galactolipid. We herein present evidence for the latter hypothesis.

## 2. Materials and methods

### 2.1. Plant material and lipid extraction

*A. thaliana* ecotype Col-0 was cultivated for 4 or 8 weeks under short day conditions [15]. Leaf discs were prepared using a cork borer (diameter 7 mm) from leaves of weeks old plants and frozen in liquid nitrogen. The leaf discs were thawed at room temperature. The lipids were extracted as described and a glycolipid fraction prepared as described [15], except that the extraction was acidified with 60 μl of acetic acid to facilitate the extraction of free fatty acids.

### 2.2. Analysis of free and bound fatty acids

The esterified fatty acids were transesterified with sodium methoxide and free fatty acids in the total extract were methylated

*Abbreviations:* 16:0, palmitic acid; 16:1, *cis*-7-hexadecenoic acid; 16:2, all *cis* 7,9-hexadecadienoic acid; 16:3, all *cis* 7,9,12-hexadecatrienoic acid; 18:2, linoleic acid, all *cis* 9,12-octadecadienoic acid; 18:3, α-linolenic acid, all *cis* 9,12,15-octadecatrienoic acid; DGDG, digalactosyl diacylglycerol; MGDG, monogalactosyl diacylglycerol; OPDA, 12-oxo-phytodienoic acid; dnOPDA, dinor-oxo-phytodienoic acid

* Corresponding authors. Fax: +46 31 786 2560 (M. Andersson).
*E-mail addresses:* mats.andersson@bioenv.gu.se (M.X. Andersson), mats.ellerstrom@gmail.com (M. Ellerström).

0014-5793/$36.00 © 2012 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.febslet.2012.06.010

with trimethylsilyl-diazomethane [14]. The fatty acid methyl esters were analyzed by GC–MS [14] using an Agilent 6820 GC and an Agilent 5975 mass selective detector operated with single ion monitoring for *m*/*z* 306.2, 278.2 and 292.2 for the methyl esters of OPDA, dnOPDA and 18:3, respectively. A calibration curve for OPDA and dnOPDA was constructed from transmethylation of purified Arabidopside E and the internal standard. OPDA and dnOPDA were detected as two isomeric peaks with the dominant representing the $\Delta^{9(13)}$-OPDA and a secondary 13-iso-OPDA [14].

### 2.3. Analysis of OPDA containing galactolipids

The glycolipid fraction obtained from 40 mg tissue was dissolved in 50 µl of methanol and 5 µl portions were analyzed by LC–MS/MS on an Agilent 1260 LC and an Agilent 6410 triple quadrupole detector. The glycolipids were separated on a 2.1 × 150 mm Prevail C18, 3 µm column with a 4 mm guard column (Alltech, Deerfield, Illinois, USA) thermostated to 30 °C. Five minutes isocratic elution with solvent A (methanol:acetonitrile:water and 12 mM ammonium formate (45:40:15, by vol.)) was followed by a linear gradient to 80% B (12 mM ammonium formate in isopropanol) in 30 min and kept for 10 min. The electrospray source was operated in negative mode with an ionization voltage of 4500 V at 300 °C with a nitrogen gas flow of 11 l/min at 40 psi. The (dn)OPDA containing lipids were detected by scanning from *m*/*z* 700 to 1300 for precursors of 291.2 (OPDA) and 263.4 (dnOPDA). The fragmentor and collision cell were operated at 150 and 15 V, respectively. The galactolipid species, and *m*/*z* for their respective formate adducts detected are listed in Table 1.

### 2.4. $^{18}$O-water incorporation and analysis

For labeling experiment, leaves were cut from 8 week old Arabidopsis plants and the petioles submerged in $^{18}$O-labeled (97% $^{18}$O, Aldrich) or unlabelled water over-night under lab lights. During the labeling the leaves transpired on average 3 times their fresh weight. The material was harvested and freeze-thawed as described above. The lipid were extracted, subjected to transmethylation and analyzed by GC–MS as above but with the additional monitoring of the 2 units heavier molecular ions. The tissue content of isotopic labeled water was assessed in a separate experiment in which leaves labeled for 4 h were dried in closed screw cap tubes at 60 °C. The tubes were cooled and a dried Hamilton syringe was dipped into the released water and inserted into the injection port of the GC. The height of the resulting 18 and 20 *m*/*z* peaks was used to calculate isotopic enrichment in tissue water.

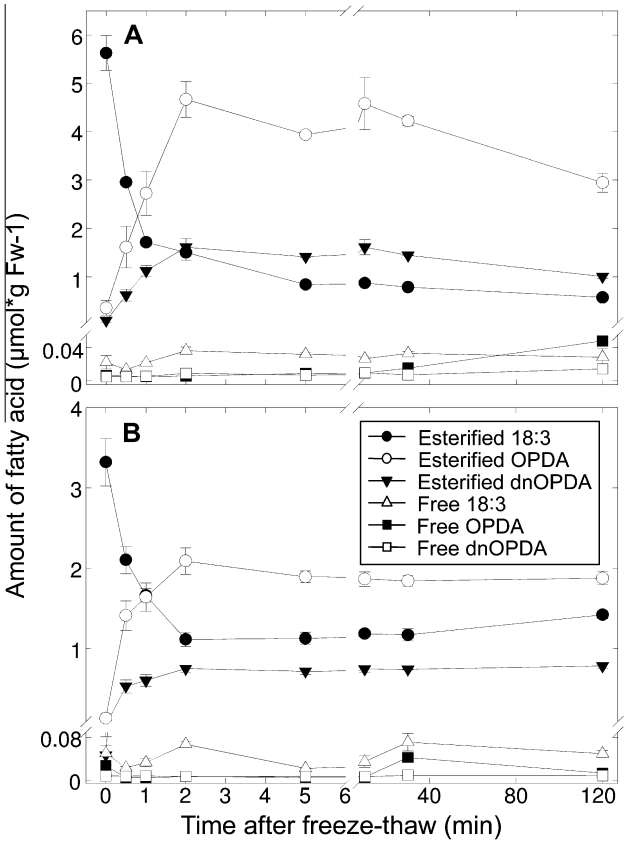


**Fig. 1.** Accumulation of esterified OPDA and dnOPDA in freeze thawed *A. thaliana* tissue. Leaf discs obtained from 8 (A) and 4 (B) weeks old *A. thaliana* were freeze–thawed and the lipids extracted at the indicated time points after thawing. The free and esterified OPDA, dnOPDA and 18:3 was quantified by GC–MS. Average and range of replicate samples are shown. Note that some of the black squares denoting free OPDA are present but obscured by other symbols.

**Table 1**
Detected OPDA containing galactolipid species. All lipids were detected as precursors of 291.2 (OPDA), except Arabidopside F which was detected as a precursor of 263.1 (dnOPDA).

| Lipid class | Fatty acids | Detected formate adduct (*m*/*z*) |
|---|---|---|
| MGDG | OPDA, dnOPDA-"Arabidopside A" | 819.5 |
| | OPDA, OPDA "Arabidopside B" | 847.6 |
| | OPDA, 16:0 | 811.6 |
| | OPDA, 16:1 | 809.6 |
| | OPDA, 16:2 | 807.6 |
| | OPDA, 16:3 | 805.6 |
| | OPDA, 18:2 | 835.6 |
| | OPDA, 18:3 | 833.6 |
| | dnOPDA, 18:3 "Arabidopside F" | 805.6 |
| DGDG | OPDA, dnOPDA "Arabidopside C" | 981.7 |
| | OPDA, OPDA "Arabidopside D" | 1009.7 |
| | OPDA, 16:0 | 973.8 |
| | OPDA, 18:3 | 995.7 |
| Acyl-MGDG | OPDA, dnOPDA, OPDA "Arabidopside E" | 1093.7 |
| | OPDA, OPDA, OPDA "Arabidopside E" | 1121.8 |
| | OPDA, dnOPDA, 16:0 | 1057.8 |
| | OPDA, dnOPDA, 18:3 | 1079.8 |
| | OPDA, dnOPDA, dnOPDA | 1065.8 |
| | OPDA, OPDA, 16:0 | 1085.8 |
| | OPDA, OPDA, 18:3 | 1107.8 |
| | 18:3, OPDA, 18:3 | 1093.8 |

## 3. Results and discussion

### 3.1. Rapid accumulation of lipid bound OPDA in tissue after loss of cellular integrity

Anecdotal evidence suggested that frozen *A. thaliana* leaf tissue thawed briefly before lipid extraction contains large amounts of glycerolipid bound OPDA (Mike Pollard, personal communication). To test this, leaf tissue from 8 weeks old *A. thaliana* was frozen in liquid nitrogen and was left to thaw for specific time periods before analysis of acyl lipid bound fatty acids by GC–MS (Fig. 1). Clearly, tissue disruption after freezing triggered massive accumulation of esterified OPDA and dnOPDA, concomitant with decrease in the content of 18:3 and 16:3 (not shown). About 4.5 µmol of OPDA and 1.8 µmol of dnOPDA were formed within the first 5 min after freeze thawing per gram tissue fresh weight (Fig. 1A). Already

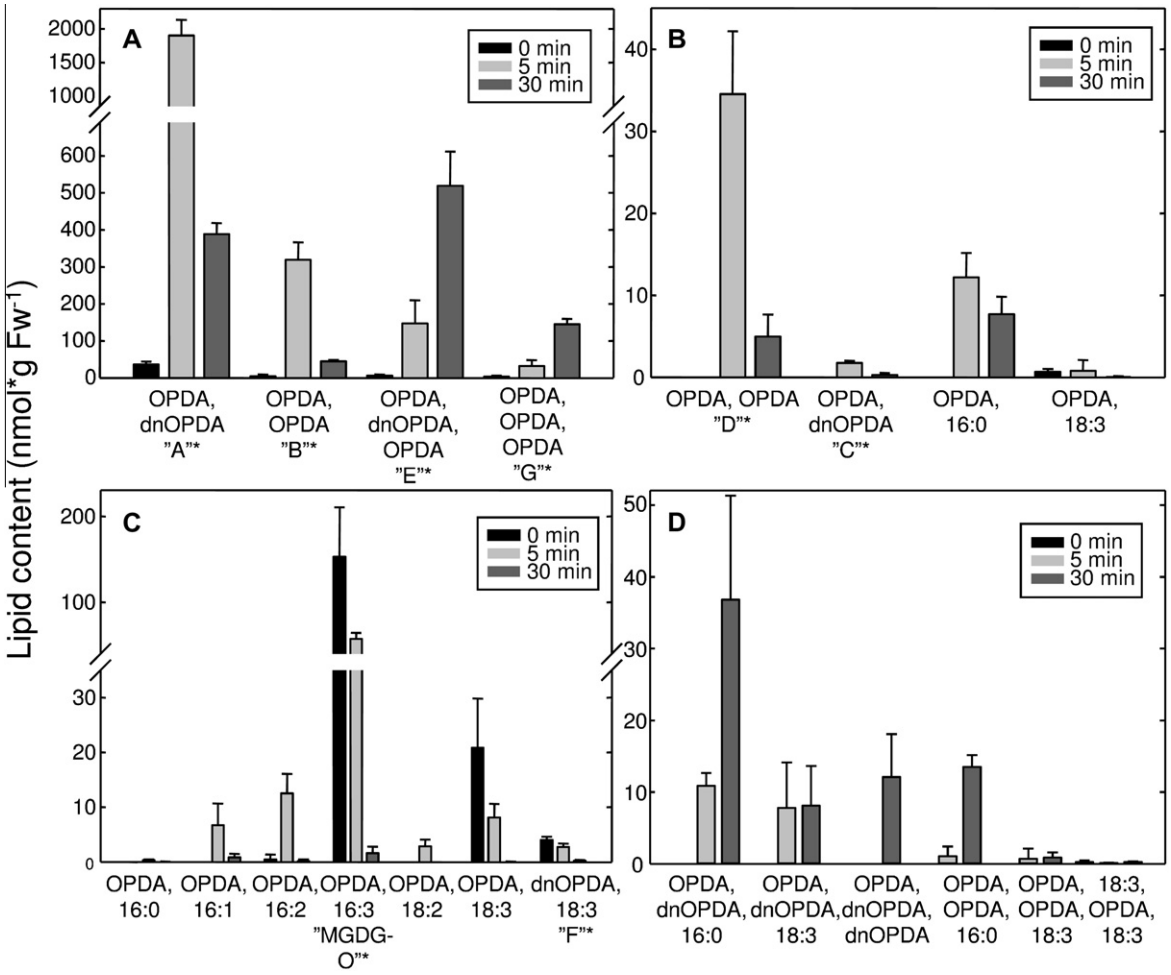


**Fig. 2.** Quantification of OPDA containing galactolipids after freeze-thawing. Leaf discs obtained from 8 weeks old *A. thaliana* leaves were freeze-thawed and the lipids extracted at the time points indicated. OPDA containing MGDG (A, C and D) and DGDG species (B) were quantified by LC–MS/MS. Species which have previously been identified as bona fide (dn)OPDA containing are marked with an asterisk and their proposed trivial names are shown below. Average and range of three replicate samples is shown.

30 s after thawing considerable amounts of OPDA and dnOPDA were formed. The amounts stabilized after 5 min and remained at the same level for 2 h. Tissue obtained from 4 week old plants contained less trienoic acids from the start and accumulated about half as much OPDA and dnOPDA as the older tissue (Fig 1B). Apparently, 60–70% of the lipid bound trienoic fatty acids in *A. thaliana* leaf tissue could be converted to (dn)OPDA rapidly after tissue disruption. Remarkably, the amount of free 18:3, OPDA and dnOPDA (Fig. 1A and B) remained constant at a very low level throughout the experiment. No esterified OPDA was detectable before or after freeze thaw in extracts from leaf tissue obtained from the AOS loss of function mutant *dde2–2* [22] (data not shown).

### 3.2. Profiling of OPDA containing lipids after freeze-thaw in Arabidopsis

Previous studies demonstrate that a large array of galactolipids containing OPDA and/or dnOPDA can be formed by various biotic and abiotic stresses [17]. Fractionation of the total lipid extract demonstrated that ~90% of the formed OPDA was associated with the glycolipid fraction (not shown). A previously used HPLC method [15] was adapted for MS/MS detection of (dn)OPDA containing galactolipids. Precursor scanning in the negative mode for ions giving rise to acyl fragments corresponding to OPDA and dnOPDA was used (Table 1). All in all, 21 different glycolipid species consistent with (dn)OPDA containing lipids were detected as formate adducts and quantified (Fig. 2). From mass alone, it cannot be unequivocally established that all these species contain OPDA and not other keto fatty acids. However, since no other keto-acids were detected by GC–MS than OPDA and dnOPDA, it is very likely that most of these lipids correspond to OPDA and/or dnOPDA containing lipids. All the detected lipid species have previously been described [8,10–18,20] and those confirmed to contain (dn)OPDA are marked with an asterisk.

In unwounded leaf tissue the major OPDA containing species was OPDA/16:3-MGDG. In abundance this was followed by OPDA/18:3-MGDG and OPDA/dnOPDA-MGDG (Arabidopside A). Five minutes after freeze thawing, there was a massive accumulation of OPDA/dnOPDA-MGDG and OPDA/OPDA-MGDG (Arabidopside A and B, respectively Fig. 2A). Other OPDA containing DGDG (Fig. 2B) and MGDG (Fig. 2C) species also increased markedly. The lipid species composition 5 min after freeze-thawing thus reflects that in unwounded tissue [23] except that the majority of the trienes are converted to (dn)OPDA. After 30 min the acylated species of OPDA containing MGDG dominated (Fig. 2A). At both analyzed time points after freeze-thawing, the four previously reported species Arabidopside A (OPDA/dnOPDA) [10], B (OPDA/OPDA) [10], E (OPDA/dnOPDA/OPDA) [14] and G (OPDA/OPDA/OPDA) [15] corresponded to 90% of the esterified OPDA. We also detected a number of putative acyl-MGDG species containing

mixes of normal fatty acids and OPDA and/or dnOPDA (Fig. 2D). Of these, the most abundant after freeze thawing was dnOPDA/OPDA/16:0. The detected OPDA containing galactolipids at 5 min after freeze-thawing corresponded to almost exactly the amount of esterified OPDA as quantified by GC–MS of methyl esters. At the 30 min time point, however, the detected species accounted for about 60% of the total esterified OPDA. This discrepancy could be explained by two different, not mutually exclusive, effects. Firstly, all quantification in LC–MS/MS is based on standard curves made from pure standards of Arabidopsides A, B, E and G. If esterified OPDA at the 30 min time point is spread on more acylated species with lower response curves, the total OPDA will be underestimated. Secondly, after acyl-transfer to the headgroup at the later time point, lyso species might have been formed. The latter would not be detected as the masses fell below the scanning range and probably elute ahead of the analyzed analytes.

### 3.3. Stable isotope labeling demonstrates formation of OPDA without release of free fatty acids

In theory, the accumulation of (dn)OPDA containing lipids could proceed through free fatty acid intermediates or directly on fatty acids that remain esterified to the lipid. The lack of increase and the consistently low levels of free trienoic acids and dn(OPDA) and the speed of accumulation of (dn)OPDA containing lipids (Figs. 1 and 2) seems less consistent with a pathway involving free intermediates. However, it cannot be ruled out that a small pool of free intermediates might be cycled quickly between lipids and free intermediates. Incorporation of $^{18}O$ from $^{18}O$-labelled water can be used to assess hydrolysis and re-acylation of fatty acids in complex lipids [24–26]. Every round of hydrolysis of fatty acids from a complex lipid results in exchange of oxygen atoms in the carboxyl group with the surrounding water. To this end, leaves were left to absorb $^{18}O$-labelled water through the petiole before freeze-thawing. Increase in isotope incorporation in (dn)OPDA containing lipids after tissue disruption would be strong evidence of that the fatty acid passes through a free intermediate, whereas lack of incorporation indicates that the fatty acid remains bound to the glycerol backbone. This is outlined schematically in Fig 3A. The methyl esters of 18:3, 18:2, OPDA and dnOPDA were analyzed by single ion monitoring of the molecular ion and the peak corresponding to the molecular ion +2 $m/z$ (incorporation of one $^{18}O$). Over-night labeling with $^{18}O$ water resulted in an increase of the $m/z$ +2 peak for all the analyzed fatty acids (Fig. 3B). The increase was largest for 18:2, which fits well with this fatty acids high turnover rate in phospholipids [27–29]. This also gives a lowest possible level of isotope enrichment in tissue water at double that of the label in 18:2, half the label is lost during transmethylation. If the 18:2 would be in isotopic equilibrium, the isotopic enrichment would be 50%. A separate experiment with a shorter labeling time demonstrated that the isotopic enrichment in the tissue water was ~50% after 4 h labeling time (data not shown). Taken together, the isotopic enrichment in the tissue water cannot have been below 50%. 18:3 and OPDA incorporated just below 10% of $^{18}O$ before freeze thawing. This clearly shows that the $^{18}O$-labeled water was not only taken up in the tissue, but was also available for incorporation into complex lipids. After freeze thawing, there was no change in the ratio of labeled to unlabeled OPDA and dnOPDA (Fig. 3B), although there was a ~20-fold increase in the amount of esterified OPDA and dnOPDA 5 and 30 min after freeze thawing (Fig. 1A). We interpret this as evidence for that the enzymatic synthesis of OPDA in Arabidopsis occurs primarily on fatty acids esterified to complex lipids. Had a significant amount of the 18:3 passed through a free intermediate between zero time and 5 min after freeze-thawing, the amount of $^{18}O$-label would approach that of 18:2 at zero time. Furthermore, the lack of increase in $^{18}O$-label

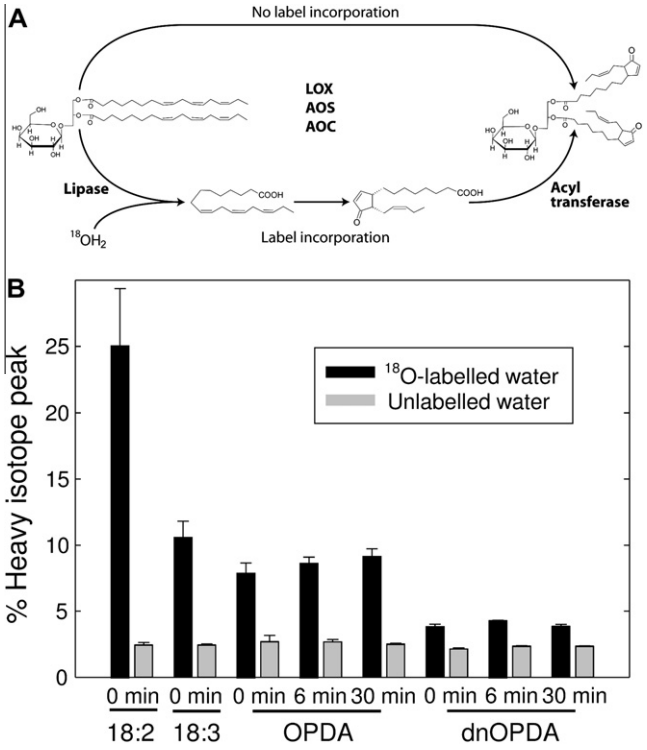


**Fig. 3.** Stable isotope incorporation into OPDA containing lipids after freeze-thawing. (A) Two possible pathways for synthesis of (dn)OPDA containing galactolipids, upper pathway: lipoxygenase (LOX), allene oxide synthase (AOS) and allene oxide cyclase (AOC) act on fatty acids esterified to the galactolipids, lower pathway: enzymes act on free fatty acids which are reacylated to the galactolipid. The lower pathway results in incorporation of $^{18}O$-labelled water into the ester bond. (B) Intact leaves from 8 week old Arabidopsis plants were fed $^{18}O$-labelled or unlabelled water through the petiole over night. The leaves were freeze-thawed and the lipids were extracted, transmethylated and analyzed by GC–MS. The ratio of labeled to unlabeled methyl esters was determined by monitoring the ratio of the unlabelled molecular ion to that of the molecular ion +2 $m/z$. Grey bars denote unlabelled and black bars labeled water. Average and range of replicate samples are shown.

between the 5 and 30 min time points is consistent with a model where fatty acids are directly transferred from the glycerol backbone of one galactolipid to the head group of another galactolipid.

To conclude, we herein present evidence for that *A. thaliana* leaf tissue is primed for the rapid conversion of trienoic acids attached to chloroplast galactolipids to OPDA and dnOPDA after loss of cellular integrity. This suggests that there is a pre-formed enzymatic machinery closely associated with the thylakoid membrane (which contains the bulk of the precursor galactolipids) that is activated after loss of cellular integrity. This is supported by the partial membrane association of LOX, AOS and AOC in potato chloroplasts [30]. However, it remains unclear what the difference is between *A. thaliana* and other plants which do not accumulate galactolipid bound (dn)OPDA. After the initial extremely fast production of lipid bound OPDA and dnOPDA, acyl transferases are activated and a distinct signature of acylated OPDA containing MGDG species is produced. While the previously described Arabidopsides A–G constitute the bulk of the (dn)OPDA containing lipids produced, there is clearly a much larger array of lipid species formed. It is still unclear how the (dn)OPDA containing galactolipids relate to synthesis of free jasmonates. It might provide a slow or delayed release of (dn)OPDA to act as a signal by itself or after conversion to JA and JA-Ile. [15]. On the other hand other observations suggest that at least the initial quick induction of JA signaling after wounding is independent of esterified OPDA production [21,31]. Thus, this seems more consistent with a role for arabidopsides in chemical defense against insects [21] or microorganisms [14,15]. Clearly,

the function is associated with dead cells and lesions in the tissue somehow. Finally, the results presented herein highlights the importance of proper immediate inactivation of enzymatic activity in plant tissues before lipid extraction to avoid artifactual synthesis of galactolipid bound (dn)OPDA.

## Acknowledgements

The financial support of the Swedish Council for Environment, Agricultural Sciences and Spatial Planning to Mats Ellerström (Project No. 2007-1051) and Mats Andersson (Project No. 2007-1563 and 2009-888), the Olle Engkvist Byggmästare foundation to Mats Andersson and the Nilsson-Ehle foundation to Anders Nilsson is gratefully acknowledged.

## References

[1] Andreou, A., Brodhun, F. and Feussner, I. (2009) Biosynthesis of oxylipins in non-mammals. Prog. Lipid Res. 48, 148–170.
[2] Browse, J. (2009) Jasmonate passes muster: a receptor and targets for the defense hormone. Annu. Rev. Plant Biol. 60, 183–205.
[3] Gfeller, A., Liechti, R. and Farmer, E.E. (2010) Arabidopsis jasmonate signaling pathway. Sci. Signal. 3 (109), cm4.
[4] Schaller, A. and Stintzi, A. (2009) Enzymes in jasmonate biosynthesis – structure, function, regulation. Phytochemistry 70, 1532–1538.
[5] Ribot, C., Zimmerli, C., Farmer, E.E., Reymond, P. and Poirier, Y. (2008) Induction of the Arabidopsis PHO1;H10 gene by 12-oxo-phytodienoic acid but not jasmonic acid via a CORONATINE INSENSITIVE1-dependent pathway. Plant Physiol. 147, 696–706.
[6] Taki, N., Sasaki-Sekimoto, Y., Obayashi, T., Kikuta, A., Kobayashi, K., Ainai, T., Yagi, K., Sakurai, N., Suzuki, H., Masuda, T., Takamiya, K., Shibata, D., Kobayashi, Y. and Ohta, H. (2005) 12-oxo-phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in Arabidopsis. Plant Physiol. 139, 1268–1283.
[7] 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.
[8] Böttcher, C. and Pollmann, S. (2009) Plant oxylipins: plant responses to 12-oxo-phytodienoic acid are governed by its specific structural and functional properties. FEBS J. 276, 4693–4704.
[9] Dave, A., Hernandez, M.L., He, Z.S., Andriotis, V.M.E., Vaistij, F.E., Larson, T.R. and Graham, I.A. (2011) 12-Oxo-phytodienoic acid accumulation during seed development represses seed germination in Arabidopsis. Plant Cell. 23, 583–599.
[10] Hisamatsu, Y., Goto, N., Hasegawa, K. and Shigemori, H. (2003) Arabidopsides A and B, two new oxylipins from *Arabidopsis thaliana*. Tetrahedron Lett. 44, 5553–5556.
[11] Hisamatsu, Y., Goto, N., Hasegawa, K. and Shigemori, H. (2006) Senescence-promoting effect of arabidopside A. Z Naturforsch. 61, 363–366.
[12] Hisamatsu, Y., Goto, N., Sekiguchi, M., Hasegawa, K. and Shigemori, H. (2005) Oxylipins arabidopsides C and D from *Arabidopsis thaliana*. J. Nat. Prod. 68, 600–603.
[13] Nakajyo, H., Hisamatsu, Y., Sekiguchi, M., Goto, N., Hasegawa, K. and Shigemori, H. (2006) Arabidopside F, a new oxylipin from *Arabidopsis thaliana*. Heterocycles 69, 295–301.
[14] Andersson, M.X., Hamberg, M., Kourtchenko, O., Brunnstrom, A., McPhail, K.L., Gerwick, W.H., Gobel, C., Feussner, I. and Ellerstrom, M. (2006) Oxylipin profiling of the hypersensitive response in *Arabidopsis thaliana* – formation of a novel oxo-phytodienoic acid-containing galactolipid, arabidopside E. J. Biol. Chem. 281, 31528–31537.
[15] Kourtchenko, O., Andersson, M.X., Hamberg, M., Brunnstrom, A., Gobel, C., McPhail, K.L., Gerwick, W.H., Feussner, I. and Ellerstrom, M. (2007) Oxo-phytodienoic acid-containing galactolipids in arabidopsis: jasmonate signaling dependence. Plant Physiol. 145, 1658–1669.
[16] Seltmann, M.A., Stingl, N.E., Lautenschlaeger, J.K., Krischke, M., Mueller, M.J. and Berger, S. (2010) Differential impact of lipoxygenase 2 and jasmonates on natural and stress-induced senescence in Arabidopsis. Plant Physiol. 152, 1940–1950.
[17] Vu, H.S., Tamura, P., Galeva, N.A., Chaturvedi, R., Roth, M.R., Williams, T.D., Wang, X.M., Shah, J. and Welti, R. (2012) Direct infusion mass spectrometry of oxylipin-containing arabidopsis membrane lipids reveals varied patterns in different stress responses. Plant Physiol. 158, 324–339.
[18] Buseman, C.M., Tamura, P., Sparks, A.A., Baughman, E.J., Maatta, S., Zhao, J., Roth, M.R., Esch, S.W., Shah, J., Williams, T.D. and Welti, R. (2006) Wounding stimulates the accumulation of glycerolipids containing oxophytodienoic acid and dinor-oxophytodienoic acid in Arabidopsis leaves. Plant Physiol. 142, 28–39.
[19] Grun, C., Berger, S., Matthes, D. and Mueller, M.J. (2007) Early accumulation of non-enzymatically synthesised oxylipins in *Arabidopsis thaliana* after infection with Pseudomonas syringae. Funct. Plant Biol. 34, 65–71.
[20] Ibrahim, A., Schutz, A.L., Galano, J.M., Herrfurth, C., Feussner, K., Durand, T., Brodhun, F. and Feussner, I. (2011) The alphabet of galactolipids in *Arabidopsis thaliana*. Front. Plant Sci. 2, 95.
[21] Glauser, G., Dubugnon, L., Mousavi, S.A., Rudaz, S., Wolfender, J.L. and Farmer, E.E. (2009) Velocity estimates for signal propagation leading to systemic jasmonic acid accumulation in wounded Arabidopsis. J. Biol. Chem. 284, 34506–34513.
[22] von Malek, B., van der Graaff, E., Schneitz, K. and Keller, B. (2002) The arabidopsis male-sterile mutant dde2-2 is defective in the ALLENE OXIDE SYNTHASE gene encoding one of the key enzymes of the jasmonic acid biosynthesis pathway. Planta 216, 187–192.
[23] Devaiah, S.P., Roth, M.R., Baughman, E., Li, M.Y., Tamura, P., Jeannotte, R., Welti, R. and Wang, X.M. (2006) Quantitative profiling of polar glycerolipid species from organs of wild-type Arabidopsis and a PHOSPHOLIPASE D alpha 1 knockout mutant. Phytochemistry 67, 1907–1924.
[24] Lombardo, D., Fanni, T., Pluckthun, A. and Dennis, E.A. (1986) Rate-determining step in phospholipase-A2 mechanism O-18 isotope exchange determined by C-13 NMR. J. Biol. Chem. 261, 1663–1666.
[25] Kuwae, T., Schmid, P.C. and Schmid, H.H.O. (1987) Assessment of phospholipid deacylation–reacylation cycles by a stable isotope technique. Biochem. Biophys. Res. Commun. 142, 86–91.
[26] Pollard, M. and Ohlrogge, J. (1999) Testing models of fatty acid transfer and lipid synthesis in spinach leaf using in vivo oxygen-18 labeling. Plant Physiol. 121, 1217–1226.
[27] Bates, P.D., Durrett, T.P., Ohlrogge, J.B. and Pollard, M. (2009) Analysis of acyl fluxes through multiple pathways of triacylglycerol synthesis in developing soybean embryos. Plant Physiol. 150, 55–72.
[28] Bates, P.D., Ohlrogge, J.B. and Pollard, M. (2007) Incorporation of newly synthesized fatty acids into cytosolic glycerolipids in pea leaves occurs via acyl editing. J. Biol. Chem. 282, 31206–31216.
[29] Benning, C. (2008) A role for lipid trafficking in chloroplast biogenesis. Prog. Lipid Res. 47, 381–389.
[30] Farmaki, T., Sanmartin, M., Jimenez, P., Paneque, M., Sanz, C., Vancanneyt, G., Leon, J. and Sanchez-Serrano, J.J. (2007) Differential distribution of the lipoxygenase pathway enzymes within potato chloroplasts. J. Exp. Bot. 58, 555–568.
[31] Koo, A.J., Gao, X., Jones, A.D. and Howe, G.A. (2009) A rapid wound signal activates the systemic synthesis of bioactive jasmonates in Arabidopsis. Plant J. Cell Mol. Biol. 59, 974–986.