Eur J Plant Pathol (2010) 127:437–442
DOI 10.1007/s10658-010-9621-1

Oxylipins are not required for *R* gene-mediated resistance in potato

Lennart Eschen-Lippold · Simone Altmann · Christiane Gebhardt · Cornelia Göbel · Ivo Feussner · Sabine Rosahl

Accepted: 19 April 2010 / Published online: 12 May 2010

Abstract The role of 9- and 13-lipoxygenase-derived oxylipins for race-cultivar-specific resistance in potato was analyzed by expressing RNA interference constructs against oxylipin biosynthetic genes in transgenic potato plants carrying the resistance gene *R1* against *Phytophthora infestans*. Down-regulation of 9-lipoxygenase expression resulted in highly reduced levels of 9-hydroxyoctadecatrienoic acid after treatment with the pathogen-associated molecular pattern Pep-13. However, neither 9-lipoxygenase nor 9-divinyl ether synthase RNAi plants exhibited alterations in their resistance to *P. infestans*. Similarly, successful down-regulation of transcript accumulation of the 13-lipoxygenase pathway genes encoding allene oxide cyclase, 12-oxophytodienoic acid reductase 3 and the jasmonic acid receptor coronatine-insensitive 1 resulted in highly reduced levels of jasmonic acid after Pep-13 treatment. Race-cultivar-specific resistance, however, was not lost in these plants. Our results suggest that neither 9-lipoxygenase-derived oxylipins nor jasmonic acid are required for *R*-gene-mediated resistance in potato. Importantly, in tobacco, the silencing of 9-lipoxygenase expression was previously demonstrated to suppress race-cultivar-specific resistance. Thus, we conclude a differential requirement of oxylipins for *R*-gene-mediated resistance in different solanaceous plants.

Keywords Divinyl ether · Jasmonic acid · Lipoxygenase pathway · *Phytophthora infestans* · Resistance · *Solanum tuberosum*

Abbreviations

9-HOT	9-hydroxyoctadecatrienoic acid
JA	Jasmonic acid
LOX	Lipoxygenase
NtLOX1	*Nicotiana tabacum* 9-lipoxygenase 1
PAMP	Pathogen-associated molecular pattern

Lennart Eschen-Lippold and Simone Altmann contributed equally to the work.

L. Eschen-Lippold · S. Altmann · S. Rosahl (✉)
Department Stress and Developmental Biology,
Leibniz Institute of Plant Biochemistry,
Weinberg 3,
06120 Halle (Saale), Germany
e-mail: srosahl@ipb-halle.de

C. Gebhardt
Department for Plant Breeding and Genetics,
Max Planck Institute for Plant Breeding Research,
Carl-von-Linné-Weg 10,
50829 Cologne, Germany

C. Göbel · I. Feussner
Department of Plant Biochemistry,
Albrecht-von-Haller-Institute for Plant Sciences,
Georg-August-University,
Justus-von-Liebig-Weg 11,
37077 Göttingen, Germany

Present Address:
S. Altmann
Department of Disease and Stress Biology,
John Innes Centre,
Norwich NR4 7UH, UK

Ppn	*Phytophthora parasitica* var. *nicotianae*
R	Resistance gene
StAOC	*Solanum tuberosum* allene oxide cyclase
StCOI1	*S. tuberosum* coronatine-insensitive 1
StLOX1	*S. tuberosum* 9-lipoxygenase 1
StDES	*S. tuberosum* 9-divinyl ether synthase
StOPR3	*S. tuberosum* 12-oxophytodienoic acid reductase 3

Upon pathogen attack, plants utilize a vast array of diverse secondary metabolites to defend themselves against infection. These metabolites can be constitutively present in the plant as preformed chemical barriers either as active compounds or inactive precursors (phytoanticipins) or are synthesized de novo upon pathogen recognition (phytoalexins; Bednarek and Osbourn 2009). Among them, directly toxic products, like the oat saponin avenacin (phytoanticipin, a triterpene glycoside) and the Arabidopsis indole alkaloid camalexin (phytoalexin) were described (Bednarek and Osbourn 2009). Additional defence-related functions are found in the class of glucosinolates. Degradation products were demonstrated to be repellent to insects, others attract predators of feeding insects or function as oviposition stimuli (Hopkins et al. 2009). Another class of defence-related secondary metabolites, the oxylipins, comprises a great variety of polyunsaturated fatty acid-derived compounds (Mosblech et al. 2009). The oxylipin pathway starts with linoleic (18:2) or α-linolenic acid (18:3) and follows two main routes depending on the first enzymatic step conducted either by 9- or 13-lipoxygenases (LOX), which introduce molecular oxygen in the carbon backbone at position 9 or 13, respectively (Andreou and Feussner 2009). In the subsequent steps, a great variety of oxylipins is generated, since at least seven different possible enzymatic branches were described (Rosahl and Feussner 2004). Some of the 9-oxylipins were shown to have direct antimicrobial properties (Weber et al. 1999; Prost et al. 2005), others are involved in signaling (Vellosillo et al. 2007). Among the 13-oxylipins, jasmonic acid (JA) functions as an important defence signal upon further modification (Browse 2009).

In several pathosystems, the involvement of 9-LOX-derived metabolites in plant defence responses has been documented. In the interactions cotton—*Xanthomonas campestris* (Jalloul et al. 2002), oilseed rape—*Hyaloperonospora brassicae* (Walters et al. 2005), Arabidopsis—*Pseudomonas syringae* (Melan et al. 1993), almond—*Aspergillus carbonarius* (Mita et al. 2007), maize—*Meloidogyne incognita* (Gao et al. 2008), tobacco—*Ralstonia solanacearum* (Cacas et al. 2005) and tobacco—*Phytophthora parasitica* (Véronési et al. 1996), the activation of the 9-LOX-branch has been shown. Interestingly, in most of these studies, a faster and more pronounced induction could be seen in incompatible interactions, when the plants are resistant due to the presence of a resistance gene (*R*), which enables the plants to specifically recognize certain races of a given pathogen (race-cultivar-specific resistance; Flor, 1971). Strikingly, in tobacco, *R* gene-mediated resistance against *P. parasitica* var. *nicotianae* (*Ppn*) has been shown to be dependent on a functional 9-LOX pathway (Rancé et al. 1998). The authors applied an antisense approach targeting the tobacco 9-LOX (*NtLOX1*), rendering the transformed plants susceptible to infection. Vice versa, susceptible plants transformed with an overexpression construct of *NtLOX1* gained elevated resistance (Mène-Saffrané et al. 2003). The *NtLOX1* product 9-hydroperoxylinole(n)ic acid is converted to colnele(n)ic acid by the 9-divinyl ether synthase *NtDES1* (Fammartino et al. 2007). This reaction might be the critical step for the establishment of the incompatibility in this plant—pathogen interaction since these divinyl ethers were demonstrated to be directly toxic to different degrees for *Phytophthora* species in vitro (Weber et al. 1999; Prost et al. 2005).

The above-mentioned activation of the 9-LOX pathway in different plant species during pathogen defence together with the specific indispensability for establishing race-cultivar-specific resistance against *Ppn* in tobacco suggest a common conserved defence mechanism. Thus, we hypothesized that products of the 9-LOX pathway, for instance 9-divinyl ethers, are the critical determinants for the expression of race-cultivar-specific resistance against *Phytophthora* species in solanaceous plants. To test this scenario in the incompatible interaction of potato with *P. infestans*, we used plants (*Solanum tuberosum* cv. Désirée) carrying the resistance gene *R1* (Ballvora et al. 2002) which are resistant against the *P. infestans* isolate 208m2 (Si-Ammour et al. 2003), carrying *Avr1* as determined with potato test lines (data not shown). The plants were transformed with RNA-interference constructs targeted against the 9-LOX *StLOX1* (= *POTLX-3*) and the 9-divinyl ether synthase *StDES*. Since we are also interested in the role of JA in pathogen defence of potato (Halim et al. 2009), we

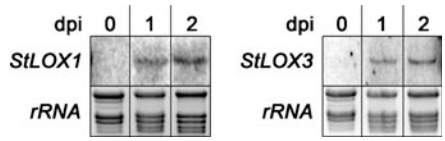

Fig. 1 LOX gene expression in *P. infestans*-infected potato *R1* plants. RNA from leaf samples of *R1* potato plants drop-inoculated with *P. infestans* ($1*10^5$ spores*ml^{-1}; harvest timepoints indicated) was subjected to Northern analyses. Hybridization was carried out with radioactively labeled probes of *StLOX1* and *StLOX3*. rRNA was stained with ethidium bromide to visualize equal loading

extended our approach to the 13-LOX pathway by silencing the JA biosynthetic genes *Allene Oxide Cyclase* (*StAOC*) and *12-Oxophytodienoic Acid Reductase 3* (*StOPR3*), as well as the JA-receptor *Coronatine Insensitive 1* (*StCOI1*) in the *R1* background. A hint for the participation of either the 9-LOX or the 13-LOX pathway in defence responses during the incompatible interaction against *P. infestans* was given by the accumulation of transcripts of both genes in response to *P. infestans* inoculation (performed as described [Eschen-Lippold et al. 2007]) in *R1* plants (Fig. 1). As demonstrated earlier, also in the susceptible interaction,

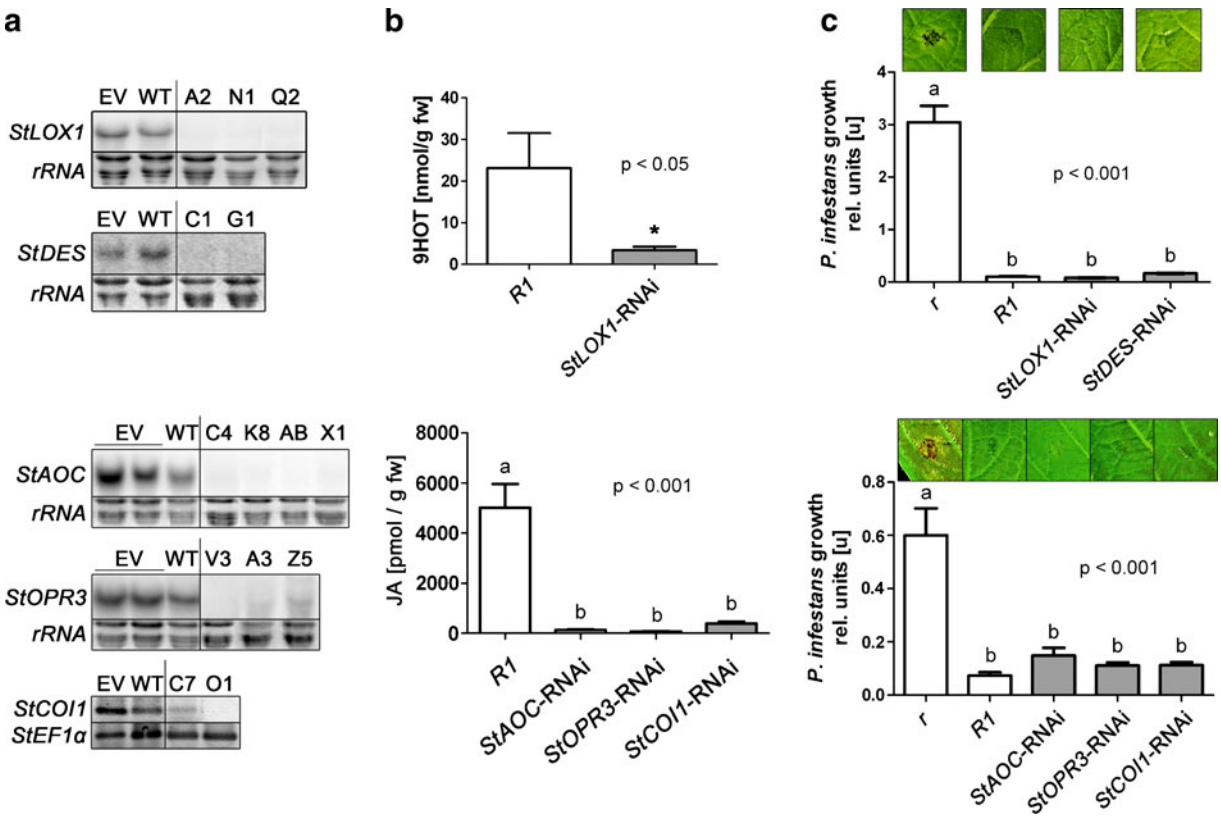

Fig. 2 Functional characterization of oxylipin pathway RNAi-plants. **a** RNA from leaves of *StLOX1*-, *StDES*-, *StAOC*-, *StOPR3*-RNAi-*R1* and control plants (EV: empty vector-carrying *R1* plants, WT: *R1* plants) infiltrated with 100 µM Pep-13 was subjected to Northern analyses. Hybridization was carried out with radioactively labelled probes of fragments corresponding to the RNAi-targeted genes. RT-PCR analysis of *StCOI1*-RNAi-*R1* plants was performed as described (Halim et al. 2009). **b** The amounts of 9-HOT and JA, respectively, were determined in leaf samples of RNAi-plants taken 24 h after infiltration of 100 µM Pep-13. *Bars* represent combined data of the respective plant lines shown in (**a**). *Asterisk* and *letters above the bars* indicate statistically significant differences (9-HOT analysis: unpaired *T*-Test, Welch's correction, $n=5$; JA analysis: one-way ANOVA, Bonferroni's multiple comparison test, $n=36$; *error bars* represent SEM). **c** Control plants (*r*: susceptible empty vector-carrying plants; *R1*: resistant *R1* plants) as well as transgenic RNAi-*R1*-plants were drop-inoculated with *P. infestans* ($1*10^5$ spores*ml^{-1}). Representative inoculation sites 3 days after infection are shown. Pathogen biomass was determined by quantitative realtime PCR 3 days after infection. *Diagrams* show data of three independent experiments each. *Bars* represent combined data of the respective plant lines shown in (**a**). *Different letters* indicate statistically significant differences (Kruskal–Wallis test, Dunn's multiple comparison test; *error bars* represent SEM)

the expression of the 9-LOX pathway genes is induced in response to *P. infestans* (Kolomiets et al. 2000; Stumpe et al. 2001; Göbel et al. 2002, 2003). However, the induction of *StLOX1* was shown to be faster in resistant potato plants (Kolomiets et al. 2000).

For our experiments, *R1*-carrying potato plants (cv. Désirée, lines 10-5 and 10–23; Ballvora et al. 2002) were transformed as previously described (Feltkamp et al. 1995) using the following RNA-interference vector constructs: to silence *StLOX1*, *StAOC*, *StOPR3* and *StCOI1*, the binary vector pHellsgate8 (Helliwell et al. 2002) was used. Since the *R1* plants were already selected on kanamycin (Ballvora et al. 2002), the *nptII*-cassette was exchanged for the hyg^R-cassette (hygromycin resistance) of the binary vector described in Gatz et al. (1992) using Bsu36I and BfrI restriction sites. The fragments of *StAOC*, *StOPR3*, *StCOI1* as well as the *StLOX1* fragment were already described (Göbel et al. 2003; Halim et al. 2009). For the *StDES*-RNAi approach, the *StDES*-RNAi vector (Eschen-Lippold et al. 2007) was modified by exchanging *nptII* with the *bar*-cassette (BASTA resistance) of the vector pGPTV-BAR (Becker et al. 1992) using HindIII and BamHI restriction sites. Positive transformants among the regenerated plants were first selected by Southern analyses (data not shown) and subsequently by Northern or RT-PCR analyses to check for downregulation of the targeted genes upon infiltration of 100 µM Pep-13 (a *Phytophthora* PAMP [*pathogen-associated molecular pattern*]; Brunner et al. 2002). Two to four independent transgenic lines reproducibly showing a reduced target gene transcript accumulation were selected for further experiments (Fig. 2a). Metabolite analyses were performed to prove the loss of accumulation of the respective compounds. Figure 2b shows the down-regulation of 9-hydroxyoctadecatrienoic acid (9-HOT) accumulation in *StLOX1*-RNAi-*R1* plants upon Pep-13 infiltration. In contrast to the more than 95% *S*-enantiomers of 9-HOT accumulating in control plants, the residual levels of 9-HOT in *StLOX1*-RNAi-*R1* plants consisted of roughly 50% *S*-enantiomers (data not shown), suggesting a non-enzymatic origin of these oxylipins. Similar reductions in enzymatically produced 9-HOT levels were also seen in *StLOX1*-RNAi plants in the susceptible background (Göbel et al. 2003). JA levels in 13-LOX pathway RNAi-lines were determined as previously described (Göbel et al. 2002). All tested lines were incapable of accumulating significant amounts of JA compared to the control plants (Fig. 2b). Thus, the RNAi approach succeeded in abolishing JA accumulation in the *R1* background. In the next step, the RNAi-*R1* plants were used for *P. infestans* 208m2 infection experiments. Phenotypically, no differences in the infection phenotype could be observed (Fig. 2c). We already showed that disease symptoms, usually recorded as lesion size, and actual pathogen spread do not necessarily correlate (Halim et al. 2007). To measure the exact *P. infestans* growth at the molecular level, we used the realtime PCR-based method described in Eschen-Lippold et al. (2007). This assay allows the direct determination of *P. infestans* biomass in the plant tissue by measuring the abundance of repetitive DNA elements. In accordance with the macroscopic phenotype, no increased susceptibility of either 9-LOX or 13-LOX pathway RNAi-*R1* lines could be detected, since all *R1*-background plants displayed the same level of resistance (Fig. 2c). Hence, the silencing of the 9-LOX branch or the 13-LOX branch of the oxylipin pathway does not lead to a break of race-cultivar-specific resistance of potato against *P. infestans*. These results differ from those obtained after down-regulation of the 9-LOX pathway in tobacco (Rancé et al. 1998). We can therefore conclude a differential requirement of the 9-LOX-derived oxylipins for race-cultivar-specific resistance against *Phytophthora* species in different solanaceous plants. Interestingly, also silencing the 13-LOX pathway had no effect on resistance in the *R1* background, although we could demonstrate the requirement of JA for the full expression of defence responses induced by Pep-13 in susceptible plants (Halim et al. 2009). Thus, JA is required for PAMP-triggered defence responses, but not for race-cultivar-specific resistance in the potato—*P. infestans* system.

Acknowledgements The excellent technical assistance of Angelika Weinel and Ulrike Smolka is gratefully acknowledged. We thank Prof. Felix Mauch for providing *P. infestans* 208m2, Sabine Freitag for help with 9-HOT analysis and Birgit Ortel for help with JA measurements. This work was funded by the Deutsche Forschungsgemeinschaft (SFB 648, TP A4).

References

Andreou, A., & Feussner, I. (2009). Lipoxygenases—structure and reaction mechanism. *Phytochemistry, 70*, 1504–1510.

Ballvora, A., Ercolano, M. R., Weiss, J., Meksem, K., Bormann, C. A., Oberhagemann, P., et al. (2002). The *R1* gene for potato resistance to late blight (*Phytophthora*

infestans) belongs to the leucine zipper/NBS/LRR class of plant resistance genes. *Plant Journal, 30*, 361–371.

Becker, D., Kemper, E., Schell, J., & Masterson, R. (1992). New plant binary vectors with selectable markers located proximal to the left T-DNA border. *Plant Molecular Biology, 20*, 1195–1197.

Bednarek, P., & Osbourn, A. (2009). Plant-microbe interactions: chemical diversity in plant defense. *Science, 324*, 746–748.

Browse, J. (2009). Jasmonate passes muster: a receptor and targets for the defense hormone. *Annual Review of Plant Biology, 60*, 183–205.

Brunner, F., Rosahl, S., Lee, J., Rudd, J. J., Geiler, C., Kauppinen, S., et al. (2002). Pep-13, a plant defense-inducing pathogen-associated pattern from *Phytophthora* transglutaminases. *EMBO Journal, 21*, 6681–6688.

Cacas, J. L., Vailleau, F., Davoine, C., Ennar, N., Agnel, J. P., Tronchet, M., et al. (2005). The combined action of 9 lipoxygenase and galactolipase is sufficient to bring about programmed cell death during tobacco hypersensitive response. *Plant Cell and Environment, 28*, 1367–1378.

Eschen-Lippold, L., Rothe, G., Stumpe, M., Göbel, C., Feussner, I., & Rosahl, S. (2007). Reduction of divinyl ether-containing polyunsaturated fatty acids in transgenic potato plants. *Phytochemistry, 68*, 797–801.

Fammartino, A., Cardinale, F., Göbel, C., Mène-Saffrané, L., Fournier, J., Feussner, I., et al. (2007). Characterization of a divinyl ether biosynthetic pathway specifically associated with pathogenesis in tobacco. *Plant Physiology, 143*, 378–388.

Feltkamp, D., Baumann, E., Schmalenbach, W., Masterson, R., & Rosahl, S. (1995). Expression of the mannopine synthase promoter in roots is dependent on the mas elements and correlates with high transcript levels of mas-binding factor. *Plant Science, 109*, 57–65.

Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annual Review of Phytopathology, 9*, 275–296.

Gao, X. Q., Starr, J., Göbel, C., Engelberth, J., Feussner, I., Tumlinson, J., et al. (2008). Maize 9-lipoxygenase *ZmLOX3* controls development, root-specific expression of defense genes, and resistance to root-knot nematodes. *Molecular Plant-Microbe Interactions, 21*, 98–109.

Gatz, C., Frohberg, C., & Wendenburg, R. (1992). Stringent repression and homogeneous de-repression by tetracycline of a modified CaMV 35S promoter in intact transgenic tobacco plants. *Plant Journal, 2*, 397–404.

Göbel, C., Feussner, I., Hamberg, M., & Rosahl, S. (2002). Oxylipin profiling in pathogen-infected potato leaves. *Biochimica et Biophysica Acta, 1584*, 55–64.

Göbel, C., Feussner, I., & Rosahl, S. (2003). Lipid peroxidation during the hypersensitive response in potato in the absence of 9-lipoxygenases. *Journal of Biological Chemistry, 278*, 52834–52840.

Halim, V. A., Eschen-Lippold, L., Altmann, S., Birschwilks, M., Scheel, D., & Rosahl, S. (2007). Salicylic acid is important for basal defense of *Solanum tuberosum* against *Phytophthora infestans*. *Molecular Plant-Microbe Interactions, 20*, 1346–1352.

Halim, V. A., Altmann, S., Ellinger, D., Eschen-Lippold, L., Miersch, O., Scheel, D., et al. (2009). PAMP-induced defense responses in potato require both salicylic acid and jasmonic acid. *Plant Journal, 57*, 230–242.

Helliwell, C. A., Wesley, S. V., Wielopolska, A. J., & Waterhouse, P. M. (2002). High-throughput vectors for efficient gene silencing in plants. *Functional Plant Biology, 29*, 1217–1225.

Hopkins, R. J., van Dam, N. M., & van Loon, J. J. A. (2009). Role of glucosinolates in insect-plant relationships and multitrophic interactions. *Annual Review of Entomology, 54*, 57–83.

Jalloul, A., Montillet, J. L., Assigbetse, K., Agnel, J. P., Delannoy, E., Triantaphylides, C., et al. (2002). Lipid peroxidation in cotton: *Xanthomonas* interactions and the role of lipoxygenases during the hypersensitive reaction. *Plant Journal, 32*, 1–12.

Kolomiets, M. V., Chen, H., Gladon, R. J., Braun, E. J., & Hannapel, D. J. (2000). A leaf lipoxygenase of potato induced specifically by pathogen infection. *Plant Physiology, 124*, 1121–1130.

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

Mène-Saffrané, L., Esquerré-Tugayé, M. T., & Fournier, J. (2003). Constitutive expression of an inducible lipoxygenase in transgenic tobacco decreases susceptibility to *Phytophthora parasitica* var. *nicotianae*. *Molecular Breeding, 12*, 271–282.

Mita, G., Fasano, P., De Domenico, S., Perrone, G., Epifani, F., Iannacone, R., et al. (2007). 9-Lipoxygenase metabolism is involved in the almond/*Aspergillus carbonarius* interaction. *Journal of Experimental Botany, 58*, 1803–1811.

Mosblech, A., Feussner, I., & Heilmann, I. (2009). Oxylipins: structurally diverse metabolites from fatty acid oxidation. *Plant Physiology and Biochemistry, 47*, 511–517.

Prost, I., Dhondt, S., Rothe, G., Vicente, J., Rodriguez, M. J., Kift, N., et al. (2005). Evaluation of the antimicrobial activities of plant oxylipins supports their involvement in defense against pathogens. *Plant Physiology, 139*, 1902–1913.

Rancé, I., Fournier, J., & Esquerré-Tugayé, M. T. (1998). The incompatible interaction between *Phytophthora parasitica* var. *nicotianae* race 0 and tobacco is suppressed in transgenic plants expressing antisense lipoxygenase sequences. *Proceedings of the National Academy of Sciences of the United States of America, 95*, 6554–6559.

Rosahl, S., & Feussner, I. (2004). Oxylipins. In D. J. Murphy (Ed.), *Plant lipids: Biology, utilisation and manipulation* (pp. 329–354). Oxford: Blackwell Publisher.

Si-Ammour, A., Mauch-Mani, B., & Mauch, F. (2003). Quantification of induced resistance against *Phytophthora* species expressing GFP as a vital marker: beta-aminobutyric acid but not BTH protects potato and Arabidopsis from infection. *Molecular Plant Pathology, 4*, 237–248.

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

Vellosillo, T., Martínez, M., López, M. A., Vicente, J., Cascón, T., Dolan, L., et al. (2007). Oxylipins produced by the 9-lipoxygenase pathway in Arabidopsis regulate lateral root development and defense responses through a specific signaling cascade. *Plant Cell, 19*, 831–846.

Véronési, C., Rickauer, M., Fournier, J., Pouénat, M. L., & Esquerré-Tugayé, M. T. (1996). Lipoxygenase gene expression in the tobacco-*Phytophthora parasitica nicotianae* interaction. *Plant Physiology, 112*, 997–1004.

Walters, D. R., Cowley, T., Weber, H., & Nashaat, N. L. (2005). Changes in oxylipins in compatible and incompatible interactions between oilseed rape and the downy mildew pathogen *Peronospora parasitica*. *Physiological and Molecular Plant Pathology, 67*, 268–273.

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