The incompatible interaction between Phytophthora parasitica var. nicotianae race 0 and tobacco is suppressed in transgenic plants expressing antisense lipoxygenase sequences

(antisense RNA/defense/signal transduction)

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Communicated by Roland Douce, University of Grenoble, Grenoble, France, March 17, 1998 (received for review December 12, 1997)

ABSTRACT Nicotiana tabacum 46-8 cultivar displays an incompatible interaction with race 0 of Phytophthora parasitica var. nicotianae (Ppn), a fungal pathogen of most tobacco cultivars. At the plant level, incompatibility is characterized by the induction of lipoxygenase (LOX, EC = 1.13.11.12) activity and localized hypersensitive cell death before defense gene activation. To evaluate the involvement of LOX in the onset of plant defense, tobacco 46-8 plants were genetically engineered using full-length or partial-length antisense (AS) tobacco LOX cDNA constructs. AS expression strongly reduced elicitor- and pathogen-induced LOX activity. Eight independent AS-LOX lines were selected and assayed for their response to Ppn. After root or stem inoculation with race 0, all AS-LOX lines but one displayed a compatible phenotype whereas control transformed plants, not containing the AS-LOX cassette, showed the typical incompatible reaction. The presence of the fungus in transgenic lines was demonstrated by PCR amplification of a Ppn-specific genomic sequence. A linear relationship was found between the extent of LOX suppression and the size of the lesion caused by the fungus. The AS-LOX plants also showed enhanced susceptibility toward the compatible fungus Rhizoctonia solani. The results demonstrate the strong involvement of LOX in the establishment of incompatibility in plant–microorganism interactions, consistent with its role in the defense of host plants.

Plant resistance to microorganisms ranges from passive and nonspecific protection involving a large set of genes to active and specific incompatibility responses in which specificity is controlled by a single gene in each partner (1, 2). In most specific systems, resistance is associated with the establishment of a hypersensitive reaction (3) and with early induction of defense gene expression. Unraveling the mechanisms underlying resistance is a major goal of plant pathology.

The development of plant resistance and the induction of defense responses are achieved by means of a complex series of events (4) arising from initial mutual recognition of the two partners and subsequent production of secondary signals. Among them, free radicals and active oxygen species might be involved in triggering hypersensitive cell death whereas ethylene, salicylic acid, and jasmonates induce defense gene expression (5). The involvement of jasmonates in plant responses to pathogens (6, 7) has reinforced our interest in plant lipoxygenases (LOXs, EC = 1.13.11.12). LOXs are non-heme dioxygenases that catalyze the oxygenation of polyunsaturated fatty acids (PUFAs), leading to the formation of hydroperoxides. In mammals, LOXs are involved in the arachidonic acid cascade that converts this PUFA into eicosanoids such as leukotrienes and lipoxins (8, 9). These products, in turn, regulate physiopathological processes, including the immune and inflammatory reactions, and the response to infectious pathogens (10). In plants, LOX gene expression is associated with a number of developmental events and induced by environmental changes, notably pathogen challenge (11). PUFA hydroperoxides resulting from the action of LOX are very reactive and may give rise to free radicals that can contribute to promoting cell death (12). The hydroperoxides may also be converted into more stable, active compounds including aldehydes and hydroxy- and epoxy-fatty acids, some of which show antimicrobial activities (13–15), and jasmonic acid (JA), derived from the LOX product 13-hydroperoxy-octadecatrienoic acid.

In the race–cultivar-specific interaction between tobacco (Nicotiana tabacum) and the phytopathogenic fungus Phytophthora parasitica var. nicotianae (Ppn), we have shown that LOX gene expression and activity are induced after root inoculation with zoospores of Ppn (16). This induction occurs as an early event in the incompatible interaction, whereas it appears 1 day later in the compatible interaction (17). Enhancement of LOX expression in response to fungal, bacterial, and viral pathogen ingress appears to be a general feature occurring both in monocots and dicots (11). In tobacco, a glycopeptide elicitor prepared from the cell wall of Ppn also induces LOX and defense gene expression as well as JA accumulation in cultured cells, whereas JA itself appears to participate in the onset of defense reactions (17, 18). Taken together, these data led to the proposal that LOX could be one of the mediators of resistance to biotic and abiotic stresses. However, its actual in vivo function in these processes is as yet unknown.

The purification of LOX from elicited tobacco cells and infected tobacco plants (16) yielded a preparation showing a single band in SDS/PAGE, suggesting that only one LOX isoform might be induced by the pathogen and its elicitors. In vitro characterization of the enzyme activity showed that it possesses both a 9- and 13-LOX specificity, with a predominance for the 9-LOX function. A cDNA encoding the enzyme specifically expressed upon infection or elicitor treatment was isolated (17, 19). To further evaluate the role of the LOX pathway in plant–pathogen interactions, we chose to modify LOX expression in tobacco via a transgenic approach by using an antisense (AS) strategy. As recipient plant, we used the tobacco cultivar 46-8, which shows resistance to race 0 of Ppn.

The present work shows the effects of AS gene expression on LOX activity and on the phenotype of tobacco plants upon inoculation with Ppn. The susceptibility of transgenic tobacco to Rhizoctonia solani, a plant pathogenic fungus with a broad host range, also was investigated.

Abbreviations: AS, antisense; JA, jasmonic acid; KanR, kanamycin resistance; LOX, lipoxygenase; Ppn, Phytophthora parasitica var. nicotianae; PUFAs, polyunsaturated fatty acid; WT, wild type.

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METHODS

Plant Material and Pathogen Isolates. Tobacco plant (Nicotiana tabacum L.) isolines 46-8 and 49-10 (20) were grown on vermiculite in the greenhouse. Line 46-8 is resistant to race 0 of Ppn whereas line 49-10 is susceptible to the same race of the fungus. Ppn race 0 was grown on oatmeal agar at 25°C in the dark. Zoospores were obtained as described (16).

Rhizoctonia solani (isolate R92-1) was propagated as a mycelial culture on sterile Malt agar medium (20 g/liter of malt extract, 2 g/liter of yeast extract) and maintained in the dark at 20°C. Seven-day-old cultures were used for inoculations of tobacco plants.

Elicitor Treatment. Elicitation was performed on 3-week-old seedlings. Plants were depotted and their roots were immediately dipped in a test tube containing either water (control) or a glycopeptide-containing elicitor preparation (30 µg/ml) obtained by autoclaving a cell wall preparation from the mycelium of Ppn (21). After absorption of the elicitor solution, plants were watered with nutrient solution and harvested 30 h after the beginning of the experiment.

Plant Inoculation. Root inoculation was carried out on 5-week-old plants with freshly prepared zoospores of Ppn race 0 (16), and symptoms were observed 5 days later. For PCR detection of the fungus in tobacco roots, a slightly modified protocol was followed. Care was taken that only the last portion (0.5 cm) of the root tips would be dipped in the zoospore suspension for 5 h. After this step, the entire root system of the plantlet was washed in sterile water, transferred to another test tube, and watered for 3 days. The inoculated portion of the root tip (0.8 cm) then was cut off and discarded to minimize possible contamination of the root material by adsorbed zoospores; the remaining root material, which had never been in contact with the zoospore suspension, was frozen for further DNA extraction.

Stem inoculations were performed on 10-week-old seedlings as described (22). The stem of each plant was sectioned 7 cm below the apex, and a mycelial pad withdrawn from the periphery of a 7-day-old colony of Ppn race 0 was immediately laid on the cut surface. The extent of fungal colonization was assessed 84 h postinoculation by measuring the length of necrotized infected tissues on a longitudinal section of the stem.

Infections with Rhizoctonia solani were conducted in a growth chamber under constant temperature (27°C), high relative humidity (100%), and with a light–dark regime of 14 h of light (220 µmol/m²-s Photons) and 10 h of dark. Mycelial discs (6 mm in diameter) were positioned upside-down on wet leaves of 10-week-old tobacco plants. Symptoms developed as radial necrosis around the mycelial disc. The fungal growth was evaluated by measuring the diameter of each necrotic lesion 4 days postinoculation. All inoculations were performed on different plants in a randomized manner, and symptoms were recorded independently by two persons.

Construction of Binary Vectors with AS-LOX Cassettes for Plant Transformation. pIPM0 was made by inserting the 420-bp fragment of the 35S RNA promoter (3'SS) of the Cauliflower Mosaic virus (23), the KpnI Small BamHI XbaI multiple cloning site (mcs) of pUC19, and the nopaline synthase terminator (tNOS) elements (Fig. 1). pIPM0, pIPM120, and pIPM125 plasmids were mobilized into Agrobacterium tumefaciens LBA4404 by using a heat-shock treatment (25). The presence and structure of the T-DNA in the bacteria were confirmed by restriction enzyme analysis and by hybridization of total DNA to the LOX cDNA probe.

Leaf disc inoculations with A. tumefaciens strains harboring pIPM0, pIPM120, or pIPM125 plasmids were carried out according to established methods (26), with axenic cultures of the tobacco 46-8 line as starting material. After 7–8 weeks on selective MS medium (150 mg/liter of kanamycin, 500 mg/liter of carbenicillin) (27), shoots were dissected away from independent transformed calli and rooted axenically in selective media without growth regulator. Transgenic plantlets then were transferred to soil in a growth chamber under constant temperature (27°C) and humidity (70%), and with a light–dark regime of 14 h of light (220 µmol/m²-s Photons) and 10 h of dark.

Screening of the T0 transformants was performed by PCR using oligonucleotide primers situated on or near the margins of the nptII and, when relevant, AS-LOX sequences. Only positive plants were transferred to the greenhouse and continued to be grown until flowering. They were allowed to self-pollinate to obtain the T1 progeny. T1 seeds were sterilized (28) and sown on solid MS medium supplemented with 200 µg/liter of kanamycin. Only the surviving green plantlets, homozygous or hemizygous for the kanamycin-resistance (KanR) trait were transferred to vermiculite and grown under the above-mentioned conditions. Elicitation and inoculation experiments were performed on these KanR plants.

Nucleic Acid Techniques. Total DNA from recombinant A. tumefaciens was extracted according to described methods (29). Plant DNA was extracted (30) and characterized by restriction enzyme digestion and Southern hybridization analysis (31).

PCR amplifications were performed in a thermocycler (Omnigene TR3, Hybaid, U.K.) by using the DNA polymerase from Thermus brockianus (ExtraPol II, Eurobio, France). Amplification reactions were conducted in 50 µl total volume containing 500 ng DNA, 10 mM Tris-HCl (pH 8.8), 50 mM KCl, 0.1% Triton X-100, 1.5 mM MgCl2, 200 µM of each dNTP, 10 pmol of each specific primer, and one unit of DNA polymerase as described (32). The cycle parameters were as follows: denaturation at 94°C for 45 sec, annealing at 63°C for 45 sec, and extension at 72°C for 1 min. The cycles were repeated 35 times, with the exception of an initial denaturation step of 5 min at 95°C and a final extension step of 5 min at 72°C.

Oligonucleotide primers corresponding to the elicitin gene of Phytophthora parasitica (33) were complementary to a portion of the promoter (5'-GCTTCTACCCACACCAC-CCC-3') and of the 3' region overlapping the end of the coding sequence (5'-CCGCTTTACGTTGCGCGCAATCG-3'). The expected amplified product was 410 bp in size.

Total RNA was extracted from tobacco following established procedures (34). Samples (20 µg each) were denatured and separated on 1% agarose gels by using the glyoxal-phosphate buffer technique (31). Northern blot hybridization was carried out in high-SDS buffer (35). The probes were radiolabeled by random priming using [α-32P]dCTP.

Protein Extraction and LOX Assays. Plant tissues were ground in liquid nitrogen and homogenized in 100 mM Tris-HCl buffer (pH 6.8) 10 mM EDTA, 5 mM DTT in the proportion of 1 ml extraction buffer per 0.5 g of tissue. The homogenate was centrifuged at 10,000 × g for 10 min at 4°C. Aliquots of the supernatant were used to measure LOX activity by polarography (16). The protein content of the crude extract was determined by the method of Bradford (36). LOX specific activity was expressed in nKat/mg protein.
Statistical Analysis of the Results. Results collected from the various experiments were analyzed by using SAS 6.11 software (37). Repeated-measures ANOVA and Tukey’s studentized range tests (HSD) were performed by using the SAS/STAT “PROC.GLM” package (SAS Institute, Cary, NC) according to the software specifications.

RESULTS

Integration of AS-LOX Cassettes into the Tobacco Genome. The previously characterized cDNA encoding an early-induced, defense-associated tobacco LOX was used to design two AS-LOX cassettes (Fig. 1). Recombinant agrobacteria bearing these constructs or the control LOX-free plasmid pIPM0 were used to transform 46-8 leaf discs. Kanamycin-resistant 46-8 independent T0 primary transformants were selected, and 10 IPM120-T0 lines, 10 IPM125-T0 lines, and 5 IPM0-T0 controls were regenerated. These transformants then were analyzed for the presence of the KanR cassette and the AS-LOX cassette, when relevant, by PCR and Southern blot experiments. Only plants containing nonarrenged copies of the constructs or the empty vector IPM0 were transferred to soil in the greenhouse for seed setting.

At all stages of development, these plants were indistinguishable from wild-type (WT) untransformed plants, notably in terms of length and size of leaves, stems, and roots; color and shape of photosynthetic tissues; number and shape of flowers; and seed content of capsules.

Further analysis of the transformants was undertaken on their T1 progeny. T1 segregation of the KanR trait was determined by sowing 500 sterilized seeds per transgenic line on selective medium. After 3 weeks, the ratio of “green KanR” to “white sowing 500 sterilized seeds per transgenic line on selective plants of the different transgenic lines in response to the elicitor according to the software specifications.

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Southern blot experiments and T1 progeny analysis allowed selection of nine unambiguous transgenic lines for subsequent experiments: four full-length IPM120 AS-LOX lines containing either one (120-2, 120-3, and 120-4) or three (120-1) T-DNA insertions; four partial-length IPM125 AS-LOX lines containing either one (125-2), two (125-4), or three (lines 125-1 and 125-3) T-DNA insertions; and one LOX-free T-DNA (IPM0) line, bearing one insertion of pIPM0, as a control.

Transgenic AS-LOX Plants Display a Dramatically Reduced LOX Activity. Because the LOX cDNA used in our study had been isolated from an elicitor-induced cDNA library of tobacco, we first determined the LOX activity of individual T1 KanR whole plants of the different transgenic lines in response to the elicitor of Ppm and compared it with the activity of nonelicited, water-treated, plants. Between four and eight seedlings per treatment and per AS-LOX transgenic line were assayed. The experiment included elicited and nonelicited controls, corresponding to WT 46-8 and KanR IPM0 plants. Fig. 2 Lower shows that the LOX activity of untreated plants was low and did not differ statistically between controls and transgenic lines. Elicitor treatment (Fig. 2 Upper), which has no visible effect on plant phenotype, provoked a 4- to 5-fold increase in LOX activity for both the 46-8 WT and IPM0 controls, indicating that there was no significant effect of transformation and regeneration on enzyme activity. In contrast, elicitor-induced LOX activity was very low in most transgenic AS-LOX plants, and, except for line 120-3, the recorded values were close to the basal LOX activity level of noninduced plants.

We then investigated the response of transgenic tobaccos to inoculation by race 0 of the fungus. This study was conducted on inoculated stems of four lines (120-1, 120-2, 120-4, and 125-1). Stem inoculation was used because it allowed easy localization of the infected area, therefore minimizing dilution by healthy tissues during extraction. Inoculated WT 46-8 plants showed a 6.8-fold increase of LOX activity over uninoculated control plants. In comparison, the LOX activity of inoculated AS-LOX lines was only induced by a factor of 2.2–4.8. Thus, the pathogen-induced enzyme activity was reduced by 30–70% compared with that of WT plants (data not shown). These differences were shown to be significant for lines 120-2, 120-4, and 125-1 and therefore confirmed the efficiency of the AS strategy. LOX transcripts and the balance between sense and AS transcript populations were quantified by Northern hybridization to the LOX cDNA probe. Total RNA from the noninoculated WT 46-8 line did not show any hybridization to this probe, whereas RNA from inoculated WT plants displayed a band at 2.9 kb corresponding to the LOX transcript (Fig. 3). In six of the eight independently transformed plants, endogenous LOX sense transcripts were not detected. Transcripts were detected in low amounts in line 120-2 (lane 4) and in higher amounts in line 120-3 (lane 5). Because of utilization of a cDNA probe and the fact that AS and sense RNAs should have the same size (2.9 kb) in the transgenic IPM120 lines, the band observed for these plants might contain either one or both populations of RNA. This was not the case for the IPM125 plant lines where the expected AS transcript (0.63 kb) was distinguishable from the 2.9-kb sense RNA.
The ability of \textit{Ppm} race 0 to colonize the roots of AS-LOX plants and differentiates sporangia as it does in control susceptible tobacco (data not shown). The fungus was undetectable in IPM0 plants and barely detectable in 120-3 plants. These results fully confirmed that AS-mediated inhibition of LOX allows \textit{Ppm} race 0 to grow inside the root system of the 46-8 tobacco cultivar in which it does not normally develop.

Transgenic Lines Have Lost the Ability to Block Pathogen Colonization in Stems. The ability of \textit{Ppm} race 0 to colonize plant tissues was quantified after stem inoculation by measuring, on longitudinal stem sections, the length of the necrotic lesions caused by the fungus 84 h postinoculation. WT-resistant plants developed a small necrotized lesion typical of a hypersensitive response, averaging 3.4 ± 0.43 mm in length (mean ± SEM, \textit{n} = 11, Fig. 5A), in which pathogen growth was arrested rapidly (Fig. 5A). In comparison, large lesions developed on susceptible plants (Fig. 5A), with an average length of 38.8 ± 1.76 mm (mean ± SEM, \textit{n} = 13, data not shown). IPM0 plants did not show statistically significant differences compared with WT-resistant plants (Fig. 5B). When transgenic AS-LOX plants were inoculated, necrotic lesions ranging from 4 to 28 mm in length could be detected in the presence of excess plant DNA (lane 3). PCR amplifications performed on template DNA extracted from inoculated tobacco isolines showed that the marker gene was not amplified from the DNA of resistant plants (lane 5). On the contrary, the elicitin sequence was amplified strongly from the DNA of susceptible tobacco roots (lane 4). Amplification carried out in the same conditions on DNA extracted from transgenic AS-LOX plants revealed that the fungus was detected in seven out of the eight transgenic lines having a resistant background but expressing an AS-LOX sequence (lanes 6–13). The fungus was not detected in line 120-3 plants, consistent with the phenotype of these plants after inoculation, and with the only slight reduction of pathogen-induced LOX activity. Histological staining of the fungus in inoculated roots with cotton blue also showed that the mycelium of \textit{Ppm} race 0 colonizes the roots of AS-LOX plants and differentiates sporangia as it does in control susceptible tobacco (data not shown). The fungus was undetectable in IPM0 plants and barely detectable in 120-3 plants. These results fully confirmed that AS-mediated inhibition of LOX allows \textit{Ppm} race 0 to grow inside the root system of the 46-8 tobacco cultivar in which it does not normally develop.

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Significant difference (Statistical analysis revealed that three lines showed a highly significant correlation between the expression of LOX and of incompatibility of the plant, and no detectable fungal growth in the roots. A linear reduction in LOX activity, no symptoms upon inoculation of the cut stem section was inoculated with a pad of Ppn race 0 mycelium. After 84 h, the stems were sectioned longitudinally and the lesions caused by the fungus were observed. From left to right: inoculated WT-susceptible 49-10 stems showing large, dark-brown rotted lesions; transgenic AS-LOX 46-8 stems (IPM125-1 retained as an example) displaying a susceptible phenotype; inoculated WT-resistant 46-8 stems, devoid of lesions and only exhibiting faint localized necrosis. (R) Measurement of stem lesions. Ten-week-old control (46-8 WT and IPM0, black bars) and transgenic (IPM120-1 to -4 and 125-1 to -4, gray bars) plants were stem-inoculated and the lesion length was measured on longitudinal stem sections. Each bar represents the size of the lesion measured on one individual. Significance was determined by repeated-measures ANOVA and Tukey’s HSD test. Significant difference from the controls: *, $P < 0.05$; **, $P < 0.001$.

**FIG. 5.** (A) Stem inoculation of WT and transgenic plants by Ppn mycelium. Ten-week-old plants were cut off 7 cm below the apex, and the cut stem section was inoculated with a pad of Ppn race 0 mycelium. After 84 h, the stems were sectioned longitudinally and the lesions caused by the fungus were observed. From left to right: inoculated WT-susceptible 49-10 stems showing large, dark-brown rotted lesions; transgenic AS-LOX 46-8 stems (IPM125-1 retained as an example) displaying a susceptible phenotype; inoculated WT-resistant 46-8 stems, devoid of lesions and only exhibiting faint localized necrosis. (R) Measurement of stem lesions. Ten-week-old control (46-8 WT and IPM0, black bars) and transgenic (IPM120-1 to -4 and 125-1 to -4, gray bars) plants were stem-inoculated and the lesion length was measured on longitudinal stem sections. Each bar represents the size of the lesion measured on one individual. Significance was determined by repeated-measures ANOVA and Tukey’s HSD test. Significant difference from the controls: *, $P < 0.05$; **, $P < 0.001$.

**FIG. 6.** Infection of control and transgenic AS-LOX lines by *Rhi zoboctonia solani*. Plants were inoculated on the leaf surface. Eight pads per plant were distributed on four different leaves. Four days postinoculation, the average lesion diameter was determined for each plant. Each bar represents the mean lesion size on a plant $\pm$ SEM. Significance was determined by repeated-measures ANOVA and Tukey’s HSD test. *, Significant difference from the WT 46-8 line at $P < 0.001$.

**DISCUSSION**

The occurrence of race–cultivar-specific interactions between plants and microorganisms offers advantages for studying the molecular events underlying resistance and defense induction in the host, allowing comparison of compatible and incompatible situations. The hypersensitive, localized cell death of the host that typifies incompatibility has proved the most efficient way for the plant to block and often destroy the pathogen. Despite recent progress in the characterization of the resistance and avirulence genes, which govern incompatibility (38), no general overview of the transduction pathways by which interaction of their products triggers the hypersensitive response has emerged. It has been proposed that lipid signals derived from the action of LOX on PUFAs might be involved in such a process. The most direct way to check this hypothesis was to produce LOX mutants and look for their phenotype upon inoculation with fungi. To this end, we chose to use an AS strategy.

The tobacco–Ppn interaction is particularly well suited for this purpose because of (i) the low number of LOX genes, probably not exceeding three in the tobacco genome, in contrast to most other plants where multiple LOX isoforms and genes have been reported; (ii) the early induction of one transcript and one isoform during incompatibility and after elicitor treatment, suggesting that only one gene is induced; (iii) the very low basal activity and expression of LOX in the vegetative organs of tobacco; and (iv) the occurrence of race–cultivar-specific interactions in this system. The cultivars studied here display clearly distinguishable phenotypes when inoculated by race 0 of the fungus: the resistant 46-8 line is devoid of externally visible symptoms, whereas the isogenic susceptible 49-10 line shows the black shank disease symptoms. The 46-8 cultivar therefore was chosen as target plant material to apply the AS strategy.

Two AS-LOX constructs were introduced into the plant genome. Examination of the resulting regenerants and T1 progeny showed that healthy transgenic plants displayed a normal phenotype, indicating that the presence of the AS-LOX transgene did not interfere with other general metabolic pathways that might have involved the other LOX enzymes. In particular, the seeds resulting from self-pollination had normal germination rates. Further analysis showed that the induction of LOX that normally occurs in response to the pathogen was decreased strongly in all transgenic lines but one, averaging values close to the basal constitutive level. This decrease was linked to the production of AS RNAs because endogenous sense LOX mRNAs were not detected in inoculated plants of most transgenic lines. Compa-
ison of transgenic AS-LOX lines with the controls showed that transgene expression resulted in a dramatically modified phenotype upon inoculation: whereas the WT 46-8 parent was symptomless, the AS-LOX 46-8 plants displayed the symptoms of a compatible interaction. Thus, this work revealed that suppressing LOX activity was sufficient to turn an incompatible phenotype into a compatible one. Statistical analysis of the data confirmed that suppression of incompatibility was effective in all transgenic lines but one, whatever the mode of inoculation, the length of the AS-LOX sequence construct, and the number of copies in the plant genome. Enhanced susceptibility was confirmed further by PCR detection of *Ppn* in transgenic plants whereas in WT-resistant plants no fungal signal was amplified. Interestingly, the transgenic line that was not impaired significantly in LOX activity also retained the incompatible phenotype. These data unequivocally demonstrate that a specific inducible LOX is essential for the expression of resistance in tobacco.

Diverse mechanisms might account for the suppression of incompatibility. Understanding whether they act directly or indirectly requires the PUFA hydroperoxides and their metabolites to be precisely determined in WT and transgenic AS-LOX plants. In tobacco, LOX gene expression is induced and JA is produced in the very first hours after elicitor treatment (17, 18). However, the production of JA is only transient, whereas induction of LOX lasts for several hours, suggesting that additional compounds are formed. One might expect incompatibility to result from the complementary effects of these diverse compounds and to be highly compromised if their levels are lowered in AS-LOX plants. Owing to the pleiotropic effects of JA when exogenously supplied to tobacco, attempts to reverse the AS phenotype by JA treatment were not undertaken.

In addition to jasmonates, a number of compounds may serve as endogenous messengers, notably hydrogen peroxide, ethylene, and salicylic acid (39, 40). The question as to whether their respective pathways share common regulatory steps and are to be precisely determined in WT and transgenic AS-LOX plants. The complementary effects of these diverse compounds and to be highly compromised if their levels are lowered in AS-LOX plants. Owing to the pleiotropic effects of JA when exogenously supplied to tobacco, attempts to reverse the AS phenotype by JA treatment were not undertaken.

In mammalians, recent reports emphasized a role of LOX products in regulating apoptotic cell death and resistance to pathogenic agents (10). These findings and the data reported in this work suggest that some proteins and functions involved in programmed cell death related to defense against pathogenic agents might be common to plants and animals. The availability of AS-LOX plants provides an efficient tool to further investigate the function of LOX in plant–microbe interactions.

We acknowledge G. Freyssinet, M.-C. Grosjean-Cournoyer, and S. Axiotis for helpful discussions, and Prof. J.-H. Weil for critical review of the manuscript. We are very much indebted to Prof. J. Lauga and Prof. J.-H. Weil for critical review of the manuscript. We are very much indebted to Prof. J. Lauga and Prof. J.-H. Weil for critical review.