A Culture Filtrate of *Phytophthora infestans* Primes Defense Reaction in Potato Cell Suspensions

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Accepted for publication 3 January 2008.

ABSTRACT

Val, F., Desender, S., Bernard, K., Potin, P., Hamelin, G., and Andrivon, D. 2007. A culture filtrate of *Phytophthora infestans* primes defense reaction in potato cell suspensions. *Phytopathology* 98:653-658.

Priming of defense reactions by an elicitor results in an enhanced ability of the plant to respond to subsequent pathogen challenges. We previously showed that application of lipopolysaccharides (LPS) to potato cell suspensions causes apoplastic acidification, but does not stimulate lipoxygenase (LOX) activity. Here, we tested the ability of various elicitors to prime and elicit defense reactions in potato cell suspensions. Adding 20 $\mu\text{g ml}^{-1}$ LPS, laminarin, harpin N, or a concentrated culture filtrate (CCF) of *Phytophthora infestans* to cell cultures 18 h before a second elicitation with LPS did not alter the intensity of apoplastic acidification compared with a single LPS application. Conversely, high

concentrations (200 or 400 $\mu\text{g ml}^{-1}$) of LPS, laminarin, and harpin N activated LOX in cells pretreated with 1 $\mu\text{g ml}^{-1}$ CCF, but not in cells pretreated with LPS, laminarin, or harpin N. LOX response was maximal in pretreated cells of potato cv. Bintje when the second elicitation occurred 18 to 24 h after CCF application. These results showed that LOX activation is primed in potato cells by CCF, but not by LPS, harpin N, or laminarin. Finally, bioassays showed a slightly greater reduction of rot weight in half tubers treated with CCF followed by LPS before inoculation with *Pectobacterium atrosepticum* than in half tubers treated with either preparation alone, indicating a priming effect of CCF on both LOX induction and disease suppression.

Additional keywords: *Solanum tuberosum*.

Priming, in plants, is a state of enhanced ability to mobilize pathogen or elicitor-induced defense responses (6). In order to set up this state, plants or cell suspensions are mostly challenged with inducers of systemic acquired resistance (SAR), such as salicylic acid (SA) and its functional analogues. Priming can also be obtained with elicitors of defense reactions used at concentrations insufficient to induce alone a measurable response (6). These primers strongly enhance a broad spectrum of cellular responses, such as an early oxidative burst, incorporation of various phenolic compounds, secretion of phytoalexins, and accumulation of defense gene products, upon subsequent challenge of pre-treated cells by avirulent bacteria (28) or by cell wall fractions from *Phytophthora sojae* (14). In parsley (*Carum petroselinum* B.), rapid K⁺/pH response and coumarin secretion were induced to a similar extent by a low dose of a 13-amino acid oligopeptide (Pep13) in SA-primed cells or by a high dose of this elicitor in nonprimed cells (13). Priming in plant cells bears some similarity with mechanisms active in primed animal and human cells. For instance, animal cells in an inflammatory state primed with p38 MAPK show increased NADPH oxidase activity and superoxide production (5), as do parsley cells primed with SA and challenged with cell wall elicitors from *P. sojae* (14).

We have previously shown (9) that lipoxygenase (LOX) activity was elicited 18 h post-challenge by both lipopolysaccharides (LPS) from *Pectobacterium atrosepticum* (Van Hall) Gardan et al. (12) (syn. *Erwinia carotovora* subsp. *atroseptica*) and *Pseudomonas corrugata* Roberts & Scarlett, and by laminarin in cell suspensions of tobacco (*Nicotiana tabacum* L. cv. BY) and

tomato (*Solanum lycopersicum* L.), but not of potato (*Solanum tuberosum* L. cvs. Bintje and Kerpondy). Interestingly, LOX activity could be triggered by a concentrated culture filtrate (CCF) of *Phytophthora infestans* in all three species, suggesting that the differential response of these species to LPS reflected their ability to respond to this specific molecule (9). We also showed that, in the few minutes following the challenge, LPS induced a significant apoplastic acidification in potato, tomato, and tobacco cell suspensions (9). This showed that LPS triggered some, but not all defense pathways in potato. LPS are major components of the outer layer of the outer membrane of gram-negative bacteria, and are known to be directly involved in the elicitation of defense reactions in a number of incompatible and compatible interactions (10). LPS have also been shown to enhance the response to subsequent bacterial attacks in pretreated plants (22). We therefore wondered whether these compounds could be acting as defense reaction primers, rather than as direct elicitors, in potato. To test this hypothesis, we challenged cell suspensions of potato cv. Bintje with low concentrations of either LPS or other potential primers (laminarin, harpin N, and a CCF of *P. infestans*), followed by a second elicitation with high concentrations of the same compounds. We tested both early and late defense reactions by measuring extracellular pH variation and lipoxygenase activity, respectively. We also investigated a priming effect on rot development on half tubers treated with CCF, LPS, or both before inoculation with *P. atrosepticum*.

MATERIALS AND METHODS

Elicitor preparation. LPS of *P. atrosepticum* (LPS_{pa}; strain CFBP 5889, INRA Angers, France) were extracted using the hot phenol-water method of Westphal and Jann (31). Lyophilized crude LPS were submitted to enzymatic treatment (Dnase I,

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Rnase, and 100 µg of proteinase K ml⁻¹) and subsequently purified by gel filtration chromatography on a Sephacryl column (20).

The CCF of *P. infestans* was prepared from a 3-week-old culture grown in sterile pea broth of a pathogenic strain (reference OEB 19 in the INRA culture collection) sampled in 2002 in Ploudaniel, France on potato cv. Bintje. The culture broth was prepared by boiling 125 g of frozen peas in 1.2 liter of ion-exchanged water, discarding the peas, and autoclaving the broth for 20 min at 120°C. The filtrate was obtained by separating the mycelium from the culture broth on 0.45 µm Whatman filter paper, dialyzed against distilled water, and lyophilized before use.

Laminarin was extracted and purified from the marine brown alga *Laminaria digitata* ([Hudson] Lamouroux) by Laboratoires Goëmar (Saint-Malo, France) as described by Klarzynski et al. (18), and supplied by the manufacturer. Harpin N (Messenger) was purchased from Eden Bioscience (Bothell, WA).

Final elicitor concentrations used for inductions were 10, 20, 200, and 400 µg ml⁻¹ for LPS_{Pa}, laminarin, and harpin N, and 1, 5, 10, and 20 µg ml⁻¹ for CCF.

Plant cell culture and culture conditions. Cell suspensions of *S. tuberosum* L. cv. Bintje were established from stems and microtubers (9). They were maintained in 80 ml of liquid, sterile MS medium at pH 5.8 (21), supplemented with sucrose (20 g liter⁻¹), 1-naphtalene-acetic acid (2 mg liter⁻¹), and 6-benzyl-aminopurine (0.5 mg liter⁻¹). Cells were grown at 25°C in the dark on a rotary shaker (130 rpm), and subcultured every 2 weeks. Elicitation experiments were performed on the day before subcultivation, with 5-ml aliquots for LOX activity or 10-ml aliquots for pH measurement.

Extracellular pH variation measurement. Variations of extracellular pH on *S. tuberosum* cv. Bintje cell suspensions were measured in 10-ml aliquots containing a magnetic shaker. The pH before elicitation was monitored every 30 s for 15 min to ensure that the observed elicitor-induced variation response was, in fact, due to elicitor treatment and not to other external factors. LPS, CCF, harpin N, or sterile-distilled water were then added to the cell suspensions at final concentrations of 10 µg ml⁻¹. The extracellular pH of the culture medium was measured with a pH electrode over 20 min, every 5 s for the first 5 min and every 30 s thereafter. After 20 min, 200 µg ml⁻¹ LPS_{Pa} was added and the pH of the culture medium was measured as described previously. Two independent experiments were performed.

Lipoxygenase activity measurement. LPS_{Pa}, laminarin, or harpin N at 20 µg ml⁻¹ or CCF at 1, 5, or 20 µg ml⁻¹ were injected in cell suspension cultures of potato cv. Bintje. After 24 h, 200 or 400 µg ml⁻¹ LPS_{Pa}, laminarin, or harpin N were added and cells were harvested and frozen after 18 h of contact. For kinetic measurements of LOX activity, 1 µg ml⁻¹ CCF (final concentration) was added to a cell suspension culture of potato cv. Bintje. After 18, 24, or 42 h, 400 µg ml⁻¹ LPS_{Pa}, 200 µg ml⁻¹ laminarin, 1 µg ml⁻¹ CCF, or water were added. Cells were then collected and frozen after 18 and 24 h. For measurement of LOX activity, cells were collected by filtration and kept at -20°C. The enzyme activity was determined as described by Bohland et al. (4), modified as follows: cells (300 mg) were ground in a mortar with 0.6 ml of cold 1 M Tris-HCl buffer, pH 6.8 containing 0.1% Triton X-100, 3 mM EDTA, 0.04% Na₂S₂O₅, and 0.1% polyvinylpyrrolidone. The homogenate was centrifuged (10,000 × g) at 5°C for 15 min. LOX activity in supernatants was measured at 234 nm on a spectrophotometer, using linolenic acid as substrate. The reaction mixture contained 100 µM linolenic acid, 0.1 M Tris buffer, pH 6.8, and 200 µl of supernatant. The increase in absorbance was followed for 15 min and the rate of increase was calculated from the initial linear proportion of the curve (initial slope). LOX activity was calculated in nkat g⁻¹ fresh weight. All experiments were performed twice with each experiment including three LOX measurements (replicates) per condition.

The priming ability of CCF on LOX activation by subsequent elicitation was assessed as ΔLOX values, calculated as the LOX activity in doubly challenged cells (1 µg ml⁻¹ CCF pretreatment for 24 h plus elicitor for 18 h), minus the sum of LOX activities in cells treated with either the elicitor alone for 18 h or CCF alone for 42 h.

Priming effect on disease development. *P. atrosepticum* cells (strain CFBP 5889, Angers, France), grown on King's B agar (Merck KGaA, Darmstadt, Germany), for 48 h in the dark at 20°C, were suspended in deionized water, and the concentration of the resulting suspension was adjusted to 10⁸ colony forming units ml⁻¹ by diluting as required after measuring optical density at 580 nm.

Inoculation bioassays were carried out on disinfected half tubers of potato cv. Bintje. A small well (5 mm deep, 5 mm diameter) was bored in the center of the cut side of each half tuber with a sterile cork borer, into which CCF (50 µl of a 20 µg ml⁻¹ aqueous solution) or water (50 µl) was deposited. After 18 h, 400 µg of LPS_{Pa} (50 µl of a 8 mg ml⁻¹ aqueous solution) or water were added in the wells and incubated for 24 h before inoculating with 50 µl of the *P. atrosepticum* suspension. After incubating for 5 days at 20°C in the dark, rotted tissue was removed from each half tuber with a small spatula and weighed (24). Five tubers per treatment were tested, and the whole experiment was performed twice. Mean rot weight was compared among treatments in a one-way analysis of variance, performed using the GLM module of the SAS 8.1 software package.

RESULTS

Acidification of extracellular medium by LPS after pre-treatment with various elicitors. No pH variation was observed in the extracellular medium of cell suspensions of potato cv. Bintje after treatment with low concentrations (10 µg ml⁻¹) of CCF, harpin N, laminarin, and LPS_{Pa} (Fig. 1). The subsequent addition of 200 µg ml⁻¹ LPS_{Pa} resulted in an acidification of extracellular medium by 0.5 pH units, which did not differ significantly (Student Newman-Keuls test, *P* = 0.05) from that induced by a single treatment with 200 µg ml⁻¹ LPS_{Pa} (Fig. 1).

Priming of LOX activity by CCF. LOX activation in potato cell suspensions pre-treated with 20 µg ml⁻¹ CCF 24 h before addition of 200 µg ml⁻¹ LPS_{Pa} was significantly higher than with LPS_{Pa} alone (200 µg ml⁻¹), but did not differ significantly from that resulting from treatment with CCF alone. LOX activation was not observed in cells pretreated with harpin N, laminarin, or LPS_{Pa} alone or upon subsequent addition of LPS_{Pa} (Fig. 2).

A significant increase in LOX activity by CCF pretreatment was observed only in cells pretreated with 1 µg ml⁻¹ CCF, but not in cells pretreated with either 5 or 20 µg ml⁻¹ CCF (Fig. 3). LOX activity was significantly higher in cells pretreated with 1 µg ml⁻¹ CCF for 24 h and subsequently challenged with LPS_{Pa}, laminarin, harpin, or CCF than in cells challenged with these elicitors without CCF pretreatment (Table 1). ΔLOX values were greater in CCF-primed cells challenged with 400 µg ml⁻¹ LPS_{Pa} or 200 µg ml⁻¹ laminarin than in cells challenged with other elicitors. These observations are surprising, since no LOX activity was observed in cells treated with either LPS or laminarin alone (Fig. 2). The priming effect with these two elicitors was statistically not different from that obtained with a double challenge with CCF (Table 1).

For all elicitors, maximum LOX activity in cells was detected 18 h after challenge with elicitor, regardless of whether challenge occurred 18 h (Fig. 4A) or 24 h (Fig. 4B) after priming with CCF. Induction of LOX activity was still detectable, although at much lower levels, when cells were challenged with elicitor 42 h after priming with CCF (Fig. 4C). Statistical comparisons of LOX activities at each time point showed no significant differences between elicitors (Student Newman-Keuls test; *P* > 0.05), except for

the elicitations 42 h after pretreatment with CCF, where laminarin caused significantly higher LOX inductions than the other three treatments. In all cases, LOX activation following the challenge was transient, with the highest value recorded 18 h after elicitation.

Effect of priming on potato soft rot symptoms. To determine if priming had an effect of disease symptom production, potato tubers were pretreated with CCF with and without LPS prior to challenge with the potato rot pathogen, *P. atrosepticum*. Although volumes of rotted tissues in tubers inoculated with *P. atrosepticum* were lower for those treated with CCF or LPS alone compared to the water-treated controls, the values were not significantly different. By contrast, half tubers treated with $1 \mu\text{g ml}^{-1}$ CCF, followed by $400 \mu\text{g ml}^{-1}$ LPS_{Pa} 18 h later and inoculated with *P. atrosepticum* developed significantly less rotted tissue than half tubers treated with LPS_{Pa} alone, CCF alone, or water (Fig. 5), indicating a priming effect of CCF on LPS-induced protection.

DISCUSSION

A clear priming of LOX activation by a low CCF concentration ($1 \mu\text{g ml}^{-1}$), but not by either laminarin, harpin N, or LPS, was observed, although there was no evidence for priming when considering changes in extracellular pH as an early defense reaction. Our results therefore suggest that priming by CCF is not dependent on early ion fluxes, since pretreatment by CCF did not alter extracellular pH variation caused by a second challenge treatment with LPS_{Pa}. This differs from observations in parsley cell cultures, where priming with SA and 2,6-dichloroisonicotinic acid (INA) led to an alteration in ion transport across the plasma membrane upon subsequent treatment with a cell wall elicitor from the oomycete *P. sojae* (7).

A priming response was only observed with the lowest concentration of CCF. The lack of priming response to a second induction with LPS when cells were pretreated with higher CCF

Fig. 1. Extracellular pH variation of cell cultures of potato cv. Bintje pretreated with $10 \mu\text{g ml}^{-1}$ lipopolysaccharide of *Pectobacterium atrosepticum* (LPS_{Pa}), harpin N, laminarin, or concentrated culture filtrate of *Phytophthora infestans* and challenged 18 h later with $200 \mu\text{g ml}^{-1}$ LPS_{Pa}. Bars are means and standard deviations of maximum pH differences in two replicate experiments.

Fig. 2. Lipoygenase (LOX) activity measured in potato cells pretreated or not with $20 \mu\text{g ml}^{-1}$ of a concentrated culture filtrate (CCF) of *Phytophthora infestans*, harpin N, laminarin, or lipopolysaccharides from *Pectobacterium atrosepticum* (LPS_{Pa}), 24 h before adding $200 \mu\text{g ml}^{-1}$ LPS_{Pa}. LOX activity was measured 18 h after the second elicitation. Bars with different letter subscripts are significantly different (Student Newman-Keuls test, $P = 0.05$).

Fig. 3. Lipoygenase (LOX) activity measured in potato cells pretreated or not with a concentrated culture filtrate (CCF) of *Phytophthora infestans* at 20, 5, or 1 µg ml⁻¹, 24 h before adding 200 µg ml⁻¹ lipopolysaccharides from *Pectobacterium atrosepticum* (LPS_{Pa}). LOX activity was measured 18 h after the second elicitation. Controls included cells treated with CCF alone (42 h after treatment) or LPS alone (18 h after treatment). Bars represent means and standard deviations of LOX activities (in nkat g⁻¹ of fresh weight), calculated over two independent experiments.

TABLE 1. Effect of priming by a concentrated culture filtrate (CCF) of *Phytophthora infestans* on lipoygenase (LOX) activity induced in potato cell suspensions challenged with different elicitors

Elicitor ^a	ΔLOX (nkat.g FW ⁻¹) ^b
LPS _{Pa} 200 µg ml ⁻¹	0.191 ± 0.084
LPS _{Pa} 400 µg ml ⁻¹	0.698 ± 0.338
Harpin N 200 µg ml ⁻¹	0.188 ± 0.019
Laminarin 200 µg ml ⁻¹	1.135 ± 0.50
CCF 1 µg ml ⁻¹	0.418 ± 0.009

^a Cells (cv. Bintje) were treated with 1 µg ml⁻¹ CCF 24 h before challenge with elicitors (200 or 400 µg ml⁻¹ LPS of *Pectobacterium atrosepticum* (LPS_{Pa}), harpin N, laminarin, or 1 µg ml⁻¹ CCF). Control cells were treated with the elicitors or CCF alone.

^b LOX activities were measured 18 h after elicitation. Because CCF at 1 µg ml⁻¹ elicits LOX activation in potato cells, priming was assessed as ΔLOX values, calculated as LOX (CCF + elicitor, 42 h) - (LOX[elicitor, 18 h] + LOX [CCF, 42 h]), where LOX (CCF + elicitor, 42 h) is the LOX activity, 42 h after addition of 1 µg ml⁻¹ CCF, in cells pre-treated with CCF for 24 h and subsequently challenged with the different elicitors, LOX (elicitor, 18 h) is the LOX activity measured in cells treated with the elicitors alone, and LOX (CCF, 42 h) is the LOX activity measured in cells treated with CCF alone. ΔLOX values indicated are means and standard deviations calculated from two independent experiments.

concentrations (≥5 µg ml⁻¹) might seem surprising at first. In this case, LOX activity initially induced by high CCF concentrations was very strong (respectively, 1.8 and 2.2 nkat g⁻¹ fresh weight 24 h after pretreatment compared with 0.5 nkat g⁻¹ fresh weight for CCF used at 1 µg ml⁻¹). Our observations are consistent with the hypothesis that priming can only be observed if LOX activity due to the pretreatment is low (or has decreased from a maximum). Since kinetic measurements revealed a transitory LOX activation peaking 18 h after treatment, in potato cells challenged with 1 µg ml⁻¹ CCF (Fig. 4), attempting second elicitation longer after the initial treatment than what we did would be useful to confirm this hypothesis.

Cells treated with CCF were able to respond to the second induction, even after 42 h when the LOX peak induced by the first CCF application is over. This suggests that there was no refractory stage for the priming effect, at variance with the report of Klarzynski et al. (18), who found a refractory state to pH priming in tobacco cells after two sequential contacts with laminarin, but not after contact with laminarin followed by oligogalacturonid.

The absence of inhibition and lack of refractory states suggest that there were no competition for recognition between the different molecules.

We showed that LOX activity can be induced in potato cells by LPS, laminarin, and even distilled water, as well as by CCF, after priming with a low CCF concentration (Fig. 4). Interestingly, the first three of these molecules are not able to induce LOX activity when used alone (9); the response primed by CCF appears thus to be highly nonspecific, and probably corresponds to the activation of metabolic pathways involved in general stress response. Previous experiments have shown that pea broth alone does not trigger LOX activity in potato cells (G. Saubeau and F. Val, *unpublished data*). We hypothesize that elicitor and priming effects within CCF result from elicitors, which are major components of *Phytophthora* culture filtrates (32). Elicitors strongly modify the structure of cell walls/membranes leading to cell death (23). These molecules share some structural and functional properties with lipid transfer proteins (3), and are able to transfer sterols between cell compartments, particularly plant cell membranes (30). It is therefore possible that these modifications could affect the perception of LPS and other molecules by treated cells (e.g., variation in osmotic potential, membrane depolarization, etc.), which in turn leads to LOX activation as a general response to these changes (11). However, it is noteworthy that a pre-treatment with cryptogin (an elicitor produced by *P. cryptogea*) makes tobacco cells less reactive to further challenges with elicitors or oligogalacturonids (2), rather than priming defense reactions. The hypothesis that the priming activity of CCF in potato cells is due to elicitors (particularly infestins) thus needs to be checked, for instance by using pure elicitors as the first elicitor.

Part of the priming process in plant cells is thought to depend on the expression of systemic acquired resistance (13,16,19,29). In this context, it is tempting to correlate the activation of LOX in potato cells by CCF perception with the activation of the jasmonic acid-pathway or with the production of other LOX-derived oxylipins. Upon CCF treatment, some lipases are probably activated and liberate free fatty acids such as linolenic acid, which will provide a substrate to activate LOX and the octadecanoid pathway (26). Potentiation of jasmonic acid-dependent responses has been reported in several plant-pathogen systems (17,15,27). Interestingly, elicitors are known to trigger systemic acquired resistance

Fig. 4. Lipooxygenase (LOX) activity induced in potato cell suspensions pretreated with concentrated culture filtrate (CCF) and challenged with various elicitor treatments at different times. The elicitors were added **A**, 18, **B**, 24, or **C**, 42 h after pretreatment (time 0). LOX activity (nkat g fresh weight) was measured at times 0, 18, and 24 h after addition of elicitor. Legend: CCF 1 $\mu\text{g ml}^{-1}$ (-----), CCF 1 $\mu\text{g ml}^{-1}$ + laminarin 200 $\mu\text{g ml}^{-1}$ ($\blacktriangle$), CCF 1 $\mu\text{g ml}^{-1}$ + LPS_{pa} 400 $\mu\text{g ml}^{-1}$ ($\blacksquare$), CCF 1 $\mu\text{g ml}^{-1}$ + water ($\times$), CCF 1 $\mu\text{g ml}^{-1}$ + CCF 1 $\mu\text{g ml}^{-1}$ ($\bullet$). LOX values shown are the means of two independent experiments.

in tobacco plants (25), and to induce SA biosynthesis in an H_2O_2 dependent way (1). The priming of LOX activity upon CCF treatment might thus result from SAR induction by elicitors present in the *P. infestans* culture filtrate.

Most priming experiments reported in the literature are based on the enhanced capacity of plants treated with biological compounds to resist subsequent pathogen attack (8). In most cases, this capacity is only assessed indirectly, by quantifying the activities of target genes or enzymes rather than by measuring symptom development (6). In this context, it is interesting to note that the priming of LOX activity by CCF observed here correlates with a decrease in the amount of symptoms in the bioassays. The data reported here are therefore good evidence that, at least in this pathosystem, the metabolic effects on key defense pathways indeed result in enhanced protection of the plant to invasion by the pathogen.

ACKNOWLEDGMENTS

We thank D. Wendehenne for very useful comments on an earlier version of this paper.

Fig. 5. Mean rot weight 5 days after inoculation in half-tubers of potato cv. Bintje treated with concentrated culture filtrate (CCF) (1 $\mu\text{g ml}^{-1}$), lipopolysaccharides from *Pectobacterium atrosepticum* (LPS_{pa}) (400 $\mu\text{g ml}^{-1}$), or CCF + LPS_{pa} before inoculation with *P. atrosepticum* CFBP 5889. Control tubers were treated with water before inoculation. Bars with different superscript letters are significantly different (Student Newman-Keuls test, $P = 0.05$).

LITERATURE CITED

- Bailleul, F., Genetet, I., and Kauffmann, S. 1996. Occurrence among *Phytophthora* species of a glycoprotein eliciting a hypersensitive response in tobacco and its relationship with elicitor. *Mol. Plant-Microbe Interact.* 9:214-216.
- Binet, M. N., Bourque, S., Lebrun-Garcia, A., Chiltz, A., and Pugin, A. 1998. Comparison of the effects of cryptogin and oligogalacturonides on tobacco cells and evidence of different forms of desensitization induced by these elicitors. *Plant Sci.* 137:33-41.
- Blein, J. P., Coutos-Thévenot, P., Marion, D., and Ponchet, M. 2002. From elicitors to lipid-transfer proteins: A new insight in cell signalling involved in plant defence mechanisms. *Trends Plant Sci.* 7:293-296.
- Bohland, C., Balkenhohl, T., Loers, G., Feussner, I., and Grambow, H. J. 1997. Differential induction of lipoxygenase isoforms in wheat upon treatment with rust fungus elicitor, chitin oligosaccharides, chitosan, and methyl jasmonate. *Plant Physiol.* 114:679-685.
- Brown, G. E., Stewart, M. Q., Bissonnette, S. A., Elia, A. E. H., Wilker, E., and Yaffe, M. B. 2004. Distinct ligand-dependent roles for p38 MAPK in priming and activation of the neutrophil NADPH oxidase. *J. Biol. Chem.* 279:7059-7068.
- Conrath, U., Pieterse, C. M. J., and Mauch-Mani, B. 2002. Priming in plant-pathogen interactions. *Trends Plant Sci.* 7:210-216.
- Conrath, U., Thulke, O., Katz, V., Schwindling, S., and Kohler, A. 2001. Priming as a mechanism in induced systemic resistance of plants. *Eur. J. Plant Pathol.* 107:113-119.
- Cools, H. J., and Ishii, H. 2002. Pre-treatment of cucumber plants with acibenzolar-S-methyl systemically primes a phenylalanine ammonia lyase gene (PAL1) for enhanced expression upon attack with a pathogenic fungus. *Physiol. Mol. Plant Pathol.* 61:273-280.
- Desender, S., Klarzynski, O., Potin, P., Barzic, M. R., Andrivon, D., and Val, F. 2006. Lipopolysaccharides of *Pectobacterium atrosepticum* and *Pseudomonas corrugata* induce different defence responses patterns in tobacco, tomato and potato. *Plant Biol.* 8:636-645.
- Erbs, G., and Newman, M. A. 2003. The role of lipopolysaccharides in induction of plant defence responses. *Mol. Plant Pathol.* 4:421-425.
- Feussner, I., and Wasternack, C. 2002. The lipoxygenase pathway. *Annu. Rev. Plant Biol.* 53:275-297.
- Gardan, L., Gouy, C., Christen, R., and Samson, R. 2003. Elevation of three subspecies of *Pectobacterium carotovorum* to species level: *Pectobacterium atrosepticum* sp. nov., *Pectobacterium betavascularum* sp. nov. and *Pectobacterium wasabiae* sp. nov. *Int. J. Syst. Evol. Microbiol.* 53:381-391.
- Katz, V., Fuchs, A., and Conrath, U. 2002. Pretreatment with salicylic acid primes parsley cells for enhanced ion transport following elicitation. *FEBS Lett.* 520:53-57.
- Katz, V. A., Thulke, O. U., and Conrath, U. 1998. A benzothiadiazole primes parsley cells for augmented elicitation of defense responses. *Plant Physiol.* 117:1333-1339.
- Kauss, H. 1994. Systemic signals condition plant cells for increased elicitation of diverse defence responses. *Biochem. Soc. Symp.* 60:95-100.

16. Kauss, H., and Jeblick, W. 1995. Pretreatment of parsley suspension cultures with salicylic acid enhances spontaneous and elicited production of H₂O₂. *Plant Physiol.* 108:1171-1178.
17. Kauss, H., Krause, K., and Jeblick, W. 1992. Methyl jasmonate conditions parsley suspension cells for increased elicitation of phenylpropanoid defense responses. *Biochem. Biophys. Res. Co.* 189:304-308.
18. Klarzynski, O., Plesse, B., Joubert, J. M., Yvin, J. C., Kopp, M., Kloareg, B., and Fritig, B. 2000. Linear beta-1,3 glucans are elicitors of defense responses in tobacco. *Plant Physiol.* 124:1027-1038.
19. Kohler, A., Schwindling, S., and Conrath, U. 2002. Benzothiadiazole-induced priming for potentiated responses to pathogen infection, wounding, and infiltration of water into leaves requires the NPR1/NIM1 gene in *Arabidopsis*. *Plant Physiol.* 128:1046-1056.
20. Meyer, A., Pühler, A., and Niehaus, K. 2001. The lipopolysaccharides of the phytopathogen *Xanthomonas campestris* pv. *campestris* induce an oxidative burst reaction in cell cultures of *Nicotiana tabacum*. *Planta* 213:214-222.
21. Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant* 15:473-497.
22. Newman, M. A., von Roepenack-Lahaye, E., Parr, A., Daniels, M. J., and Dow, M. 2002. Prior exposure to lipopolysaccharide potentiates expression of plant defenses in response to bacteria. *Plant J.* 29:487-495.
23. Panabières, F., Birch, P. R. J., Unkles, S. E., Ponchet, M., Lacourt, I., Venard, P., Keller, H., Allasia, V., Ricci, P., and Duncan, J. 1998. Heterologous expression of a basic elicitor from *Phytophthora cryptogea* in *Phytophthora infestans* increased its ability to cause leaf necrosis in tobacco. *Microbiology* 144:3343-3349.
24. Pasco, C., Bozec, M., Ellissèche, D., and Andrivon, D. 2006. Resistance behaviour of potato cultivars and advanced breeding clones to tuber soft rot caused by *Pectobacterium atrosepticum*. *Potato Res.* 49:91-98.
25. Ricci, P., Panabières, F., Bonnet, P., Maia, N., Ponchet, M., Devergne, J. C., Marais, A., Cardin, L., Milat, M. L., and Blein, J. P. 1993. Proteinaceous elicitors of plant defence responses. Pages 121-135 in: *Mechanisms of Plant Defence Responses*. B. Fritig and M. Legrand, eds. Kluwer Academic Publishers, Dordrecht, The Netherlands.
26. Schaller, F. 2001. Enzymes of the biosynthesis of octadecanoid-derived signalling molecules. *J. Exp. Bot.* 52:11-23.
27. Schweizer, P., Buchala, A., and Metraux, J. P. 1997. Gene-expression patterns and levels of jasmonic acid in rice treated with the resistance inducer 2,6-dichloroisonicotinic acid. *Plant Physiol.* 115:61-70.
28. Shirasu, K., Nakajima, H., Rajasekhar, V. K., Dixon, R. A., and Lamb, C. 1997. Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signals in the activation of defense mechanisms. *Plant Cell* 9:261-270.
29. Ton, J., and Mauch-Mani, B. 2004. β -Amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. *Plant J.* 30:119-130.
30. Vauthrin, S., Mikes, V., Milat, M. L., Ponchet, M., Maume, B., Osman, H., and Blein, J. P. 1999. Elicitins trap and transfer sterol from micelles, liposome and plant plasma membranes. *Biochim. Biophys. Acta* 1419: 335-342.
31. Westphal, O., and Jann, K. 1965. Bacterial lipopolysaccharides: Extraction with phenol-water and further application of the procedure. *Carbohydr. Chem.* 5:83-91.
32. Yu, L. M. 1995. Elicitins from *Phytophthora* and basic resistance in tobacco. *Proc. Natl. Acad. Sci. USA* 92:4088-4094.