# A pathogen-inducible divinyl ether synthase (*CYP74D*) from elicitor-treated potato suspension cells[1]

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**Abstract In elicitor-treated potato cells, 9-lipoxygenase-derived oxylipins accumulate with the divinyl ether colneleic acid as the major metabolite. Here, the identification of a potato cDNA is described, whose predicted amino acid sequence corresponds to divinyl ether synthases, belonging to the recently identified new P450 subfamily CYP74D. The recombinant protein was expressed in *Escherichia coli* and shown to metabolize 9-hydroperoxy linoleic acid to colneleic acid at pH 6.5. This fatty acid derivative has been implicated in functioning as a plant antimicrobial compound. RNA blot analyses revealed accumulation of divinyl ether synthase transcripts both upon infiltration of potato leaves with *Pseudomonas syringae* and after infection with *Phytophthora infestans*.** 

*Key words:* Substrate specificity; *CYP74D*; *Solanum tuberosum*; *Phytophthora infestans*; *Pseudomonas syringae*

## 1. Introduction

The formation of fatty acid hydroperoxides from polyunsaturated fatty acids is the first step in the synthesis of oxidized polyenoic fatty acids, named oxylipins [1]. Their biosynthesis may either occur by autoxidation or by the action of enzymes, such as lipoxygenase (LOX) or α-dioxygenase [2]. The metabolism of polyenoic fatty acids via the LOX reaction is collectively named LOX pathway, and the catabolism of LOX-derived fatty acid hydroperoxides is a central branching point forming its own metabolic network. In plants, up to seven different enzyme families compete for LOX-derived hydroperoxy derivatives as substrates [2]. At least four major metabolic routes have been characterized in more detail [1,3]: (i) The hydroperoxide lyase (HPL) pathway: here, the hydrocarbon backbone of fatty acid hydroperoxides is cleaved under rearrangement of the hydroperoxide leading to the formation of short chain aldehydes (C6- or C9-) and the corresponding C12- or C9-ω-keto fatty acids [4]. (ii) The allene oxide synthase (AOS) pathway, where hydroperoxy fatty acids are converted to unstable allene oxides, which either form non-enzymatically racemic derivatives of 12-oxo phytodienoic acid or undergo non-enzymatic hydrolysis forming α- and γ-ketols [5]. In the presence of allene oxide cyclase, the allene oxide is metabolized to (9*S*,13*S*)-12-oxo phytodienoic acid [6]. (iii) The peroxygenase pathway: within this pathway, intramolecular oxygen transfer converts fatty acid hydroperoxides to epoxy- or dihydrodiol polyenoic fatty acids [1,7]. (iv) The recently described divinyl ether synthase (DES) reaction [8]: this pathway converts fatty acid hydroperoxides into cytotoxic divinyl ethers [9].

Of these four enzyme families, at least three form a distinct subfamily based on protein sequence similarities and biochemical properties. These three belong to a family of cytochrome P450s, named *CYP74* [8]. The *CYP74A* subfamily consists of enzymes exhibiting AOS activity while those enzymes with HPL activity are divided in two subgroups based on their substrate preference. Thus, the *CYP74B* subfamily contains HPLs acting on 13-hydroperoxides (13-HPLs), and HPLs which accept either 9- or 13-hydroperoxides as substrates (9/13-HPLs) are grouped into the subfamily CYP74C. Recently, enzymes with DES activity have been grouped into the *CYP74D* subfamily. *CYP74* are unique among other P450 monooxygenases in that they do not require molecular oxygen nor NADPH-dependent cytochrome P450 reductase as cofactors [4,5]. While AOS and HPL isozymes exist which accept either the C13- or C9-hydroperoxy derivatives (9/13-AOS or 9/13-HPL, respectively), the only DES enzyme described so far from tomato is strictly dependent on the C9 derivatives [8]. This is in accordance with studies in potato where the endogenously occurring divinyl ethers called colneleic acid (CA) or colnelenic acid (CnA) have been suggested to be derived from linoleic acid or linolenic acid, respectively, by the sequential action of a 9-LOX and a 9-DES [10]. Most interestingly, recent findings suggest that these 9-LOX-derived oxylipins may play an important function in the defense of solanaceous plants against pathogens, because reports provide evidence that both CA and CnA have antimicrobial activity against *Phytophthora infestans* [9], and a more detailed inspection of oxylipins induced in elicitor-treated potato cells revealed that CA was indeed the main oxylipin accumulating in these cells [11]. In addition to these correlative data, the importance of 9-LOX in conferring resistance against pathogens was demonstrated in tobacco plants in which the activity of a specific 9-LOX was inhibited by expression of its antisense construct [12]. In contrast to resistant wild-type plants, the transgenic plants became susceptible to infection with *Phytophthora parasitica*. Such a conversion of an incompatible

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interaction into a compatible one suggests a crucial role for 9-LOX-derived oxylipins in conferring resistance in solanaceous plants. In order to study this resistance response in more detail, we started to analyze the biosynthesis of divinyl ether fatty acids by DES in potato by isolating its cDNA for further characterization of its biochemical and molecular properties.

## 2. Materials and methods

### 2.1. cDNA isolation

RNA was isolated and analyzed as described [13]. RNA from suspension-cultured potato cells was isolated as described [14]. From total RNA, mRNA was isolated by using the PolyATtract® Kit (Promega, Mannheim, Germany). First-strand cDNA was generated from mRNA of potato cell suspension cultures by reversed transcription with SuperscriptII (Gibco BRL, Eggenstein, Germany). This ss-cDNA was used as template for PCR-based cloning. A 450 bp PCR fragment was amplified using primers homologous to an EST sequence from tomato (TIGR database EST281141), sense primer A 5′-AAT CCG TCT GAT ACA GTT CTT GGC GC-3′ and antisense primer B 5′-CGA TTT GAC CAA ACT CAT TTT GTT AAT CG-3′. The PCR reaction was carried out with TfI-DNA-Polymerase (Biozym, Hess. Oldendorf, Germany) using an amplification program of 2 min denaturation at 94°C, followed by 10 cycles of 30 s at 94°C, 45 s at 51°C, 45 s at 72°C, followed by 20 cycles of 30 s at 94°C, 45 s at 51°C, 45 s at 72°C (time increment 3 s) and terminated by 10 min extension at 72°C. PCR products of the expected length were cloned in pCR2.1-TOPO (Invitrogen, Karlsruhe, Germany) and sequenced. The fragment StDes was chosen for the isolation of a DES-encoding cDNA clone using the SMART™ RACE cDNA amplification kit (Clontech, Heidelberg, Germany). To amplify the 5′- and 3′-ends of StDes by PCR, specific primers were used: 5′-RACE: primer C 5′-GCT TCA TCC AAT ATT GAC ACA GCG GAC-3′, 3′-RACE: primer D 5′-GTC CGC TGT GTC AAT ATT GGA TGA AGC-3′. The PCR reaction was carried out with TfI-DNA-Polymerase using an amplification program of 1 min denaturation at 94°C, followed by 10 cycles of 30 s at 94°C, 30 s at 58°C, 2 min at 72°C, followed by 20 cycles of 30 s at 94°C, 30 s at 55°C, 2 min at 72°C and terminated by 3 min extension at 72°C. The 3′-fragment of 800 bp was of the expected size and cloned in pGEM®-T Easy (Promega, Mannheim, Germany) and sequenced. In case of the 5′-RACE, no fragment of the expected size was obtained. To obtain the complete cDNA clone by PCR, specific primers of the expected open reading frame of the entire cDNA either derived from the isolated 3′-RACE fragment and in case of the 5′-end from the sequence of the DES from tomato [8] were used for amplification: sense primer E 5′-GGA TCC ATG TCT TCT TAT TCA GAG CTA TCA AAT C-3′ and antisense primer F 5′-CCC AAG CTT CTA TTT ACT TGC TTT GGT TAA CG-3′. The PCR reaction was carried out with the expand high fidelity system (Roche, Mannheim, Germany) using an amplification program of 2 min denaturation at 94°C, followed by 10 cycles of 30 s at 94°C, 30 s at 61°C, 1.5 min at 72°C, followed by 20 cycles of 30 s at 94°C, 30 s at 64°C, 1.5 min at 72°C (time increment 5 s) and terminated by 2 min extension at 72°C. The fragment was cloned into pGEM®-T Easy and the resulting plasmid pStDES was sequenced.

### 2.2. Expression of recombinant StDES

For expression in *Escherichia coli* the open reading frame of pStDES was cloned as a *Bam*HI/*Hin*dIII fragment into the shuttle vector pQE30 (Qiagen, Hilden, Germany) to yield the plasmid pQEStDES. The plasmid pQEStDES was transformed into the *E. coli* strain SG13009[pRep4] (Qiagen, Hilden, Germany). The expression of DES in cells harboring the plasmid pQEStDES was performed essentially as described before with the exception that the cultivation was performed at 10°C for 24 h [15].

For purification of the recombinant protein, cells were harvested and spun down by centrifugation for 15 min at 4500 rpm and 4°C. Then the pellet was suspended in 25 ml of 50 mM sodium phosphate buffer, pH 8.0, and cells were broken by using a sonifier tip five times each for pulses of 1 min on ice. The cell debris was separated by another centrifugation for 15 min at 4000 rpm and 4°C and the membrane fraction was isolated by a third centrifugation at 100 000×*g* for 1 h at 4°C. The resulting pellet was resolved by an incubation for 1 h on ice in 10 ml of 50 mM sodium phosphate buffer, pH 8.0, containing 1 M NaCl and 0.1% (v/v) Triton X-100. Insoluble protein was separated by ultracentrifugation at 100 000×*g* for 1 h at 4°C. The resulting supernatant was incubated overnight at 4°C with 2 ml of TALON™ Resin (Clontech, Heidelberg, Germany), which was equilibrated in 50 mM of sodium phosphate buffer, pH 8.0, containing 1 M NaCl. Then the suspension was passed into a *Bakerbond filtration column* (T.J. Baker, Phillipsburg, USA) of 3 ml and column material sequentially washed with 20 ml of sodium phosphate buffer (50 mM, pH 8.0, containing 1 M NaCl), 7 ml of sodium phosphate buffer (50 mM, pH 7.0, containing 1 M NaCl), 4 ml of sodium phosphate buffer (50 mM, pH 6.0, containing 1 M NaCl) and 2 ml of sodium phosphate buffer (50 mM, pH 5.0, containing 1 M NaCl). The active and pure protein was then eluted with 2 ml of sodium phosphate buffer (50 mM, pH 4.0, containing 1 M NaCl) with a yield of about 100 μg/l of bacterial culture.

### 2.3. Enzyme activity tests

The fatty acid hydroperoxides (13-HPOT, 13-HPOD, 9-HPOT and 9-HPOD) were prepared by incubation of the corresponding fatty acids either with soybean 13-LOX (Sigma, Germany) or for the 9-LOX-derived hydroperoxides (9-HPOD and 9-HPOT) by incubation of the appropriate precursor fatty acids with recombinant potato tuber LOX [13]. Linoleic acid and linolenic acid were obtained from Cayman Chemicals (Ann Arbor, USA) and divinyl ether fatty acids (standards) were from Larodan (Vlalmö, Sweden).

### 2.4. Product analysis

2 μg affinity-purified recombinant potato DES diluted in 0.9 ml 50 mM sodium phosphate buffer, pH 6.5, were incubated with 100 nmol of 9-HPOD for 30 min at room temperature. The reaction was stopped by adding 100 μl of glacial acetic acid and extracted as described [16]. The combined organic phases were evaporated under a stream of nitrogen and lipids were reconstituted in 100 μl of HPLC solvent (acetonitrile:water:acetic acid, 60:40:0.1 v/v/v). The HPLC analysis was essentially performed as described before [17] with an Agilent 1100 HPLC system (Waldbronn, Germany). For detection of vinyl ethers the absorbance at 252 nm was recorded. An authentic standard of colneleic acid eluted at about 31 min. Alternatively, the corresponding fatty acid methyl esters were analyzed by gas chromatography/mass spectrometry (GC/MS). For derivatization, the free fatty acid derivatives were solved in 400 μl of methanol and incubated with 10 μl of an 1-ethyl-3(3-dimethylaminopropyl) carbodiimide hydrochloride stock solution (10 mg in 100 μl of methanol) for 2 h at room temperature. Then 200 μl of Tris–HCl (0.1 M, pH 7.5) was added and the formed fatty acid methyl esters were twice extracted with 1 ml of hexane. GC/MS analysis was performed with an Agilent GC 6890 system coupled with an Agilent 5973N MS detector. The GC was equipped with a capillary HP-5 column (5% diphenyl–95% polydimethyl siloxane, 30 m×0.25 mm; 0.25 μm coating thickness; Agilent, Waldbronn, Germany). Helium was used as carrier gas (40 cm/s). An electron energy of 70 eV, an ion source temperature of 230°C, and a temperature of 275°C for the transfer line was used. The samples were measured in the EI mode, and the splitless injection mode (opened after 1 min) with an injector temperature of 250°C. The temperature gradient was 60–110°C at 25°C/min, 110°C for 1 min, 110–270°C at 10°C/min, and 270°C for 10 min. A standard of CA eluted with a retention time of 17 min.

### 2.5. Northern blot analysis

Leaves of 5 weeks old potato plants (*Solanum tuberosum* L. cv. Désirée) grown from sterile plants in a phytochamber (16 h light [200 μE], 18°C, 60% humidity) were infiltrated with $10^8$ CFU of *Pseudomonas syringae* pv. *maculicola* or infected with a suspension of $10^5$/ml zoospores of *P. infestans*. Total RNA was separated on formaldehyde gels and transferred to nylon membranes (Roche, Mannheim, Germany). cDNA fragments were labeled using the Mega Prime DNA labeling system (Amersham Pharmacia Biotechnology, Freiburg, Germany) and hybridization was carried out in 5×SSPE, 5×Denhardt's, 0.1% SDS, 50% formamide, 100 μg/ml denatured salmon sperm DNA. Filters were washed three times at 60°C with 3×SSC, 0.1% SDS. As probes, a 1.4 kb *Eco*RI fragment of StLOX1 [14], the 1.4 kb fragment from the DES cDNA, a 2.0 kb *Eco*RI fragment from St4CL [18] and a 1.3 kb *Bam*HI fragment from St25S rRNA (kindly provided by J. Petters) were used.

## 3. Results

### 3.1. PCR-based cloning and isolation of a DES-encoding cDNA of StDES from elicited potato suspension cells

In order to analyze the biosynthesis of divinyl ether fatty acids by DES in potato, we first isolated its cDNA by PCR. For that purpose we designed degenerate primers from an EST from tomato (TIGR database EST281141) which was annotated as coding for an AOS, but which showed only moderate homology to its isozymes in tomato. The templates used were first-strand cDNA samples generated from RNA of stored potato tubers. However, using these cDNA probes, all attempts to isolate cDNA fragments coding for *CYP74* enzymes were without success. In contrast, when we used cDNA isolated from elicitor-treated potato cell suspension cultures, we were able to isolate a fragment of the expected size. The isolated PCR product was sequenced and showed 95% homology to the corresponding EST from tomato. To isolate the DES-encoding cDNA, 3′- and 5′-RACE experiments were carried out. In the 3′-RACE experiment, the complete fragment of about 800 bp was isolated, but all attempts to isolate the complete fragment by 5′-RACE failed. During that time the identification of a DES from tomato was pre-published in the internet [8], and we therefore designed a homologous primer for the 5′-end derived from that sequence. Using this primer and a primer derived from the 3′-RACE fragment, we amplified a fragment of the size of the expected DES-encoding cDNA by PCR. The fragment StDES was cloned into pGEM-T Easy and sequenced. The isolated DES-encoding cDNA had a length of 1437 bp encoding a protein of 478 amino acids. This *CYP74* sequence showed highest identity to the DES from tomato (96%) [8], the 9-HPL of melon (50%) [19], and the 9-HPL of cucumber (49%) [20]. These findings were further supported by phylogenetic tree analysis. As shown in Fig. 1 the newly identified DES from potato groups with the 9-DES from tomato into one subfamily. Most interestingly, these 9-DESs form together with the 9-HPLs a subfamily, whereas 13-AOS, 13-HPL, and 9-AOS form separate subfamilies [20].

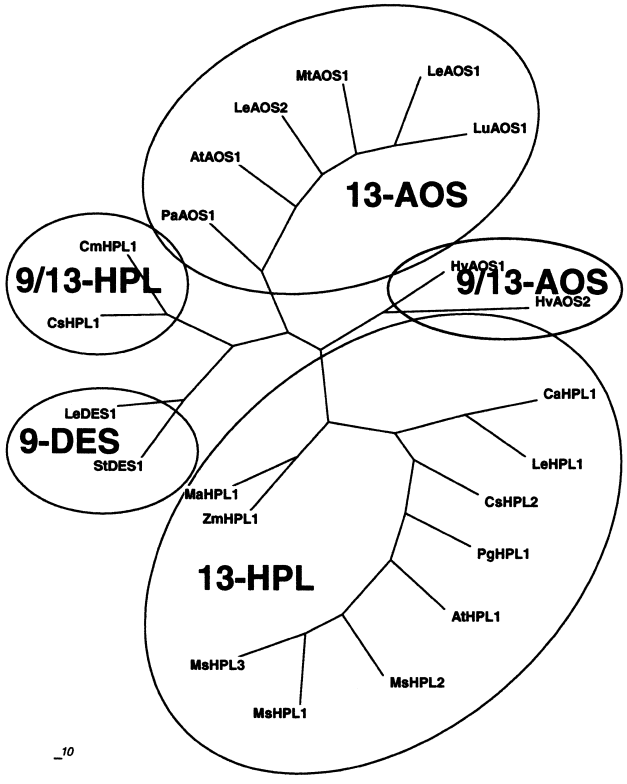


Fig. 1. Phylogenetic tree analysis of the *CYP74* enzyme family. The analysis was performed with the program Phylip 3.5. cDNAs corresponding to the following accession numbers were used for the analysis: MsHPL1: CAB54847, MsHPL2: CAB54848, MsHPL3: CAB54849, AtHPL1: AAC69871, LeHPL1: AAF67142, CaHPL1: AAA97465, MaHPL1: CAB39331, ZmHPL1: from patent WO00/22145, CsHPL1: AAF64041, PgHPL1: AAK15070, StDES: CAC28152, LeDES: AAG42261, HvAOS1: CAB86384, HvAOS2: CAB86383, PaAOS1: CAA55025, LeAOS1: CAB88032, LeAOS2: AAF67141, AtAOS: CAA63266, LuAOS: AAA03353, CsHPL2: AF229812, MtAOS1: TC22359, CmHPL1: AAK54282.

### 3.2. Functional expression in E. coli and product analysis

In order to characterize the biochemical properties of the cDNA, we cloned it into a His-tag expression vector and expressed the protein in *E. coli*. The pH optimum of the purified enzyme was determined spectrophotometrically with (9*S*)-hydroperoxy linoleic acid (9-HPOD) as a substrate and turned out to be rather broad between pH 5.5 and 7.5 (data not shown). Furthermore, when the 9-hydroperoxy derivatives of linoleic acid and linolenic acid were compared as substrates, linolenic acid was metabolized at a lower rate of about 50%. The corresponding 13-hydroperoxy derivatives were no substrates of the enzyme. The $K_m$ and $V_{max}$ values were determined to be 17.4 μM and 5.3 U/mg, respectively, for 9-HPOD and 26.1 μM and 2.8 U/mg, respectively, for 9-HPOT. To characterize the products of this enzyme reaction in more detail, we analyzed the compounds formed by incubation of recombinant StDES with 9-HPOD at pH 6.5 by HPLC analysis (Fig. 2A) and by GC/MS analysis (Fig. 2B). As shown in Fig. 2A, the product with a retention time of 31 min could be clearly separated from the substrate 9-HPOD, eluting at a retention time of 16 min. The UV spectrum of this compound is shown as an inset in Fig. 2A, revealing a strong UV absorption with a $\lambda_{max}$ at 250 nm, as has been described for CA [8,10]. Subsequently, GC/MS was used to identify the methylated derivative of the substance eluting at 16 min presumably being CA. This substance showed one major peak in the GC chromatogram eluting at about 17 min and the mass spectrum recorded at this position is shown in Fig. 2B. It showed as prominent ion at a *m*/*z* of 308 ($M^+$) the expected molecular mass of CA. Moreover, the fragmentation pattern of this spectrum was in agreement with earlier published spectra on the methyl ester of CA [8,10].

### 3.3. Pathogen-induced StDES transcript accumulation

Since both CA and CnA accumulate in potato leaves infected by *P. infestans* [9] and since CA has been identified as the major oxylipin in elicitor-treated potato cells [11], we analyzed whether 9-LOX (*LOX1*) and *DES* transcript accumulation is pathogen-induced in potato. RNA analyses were carried out with potato plants infiltrated with the phytopathogenic bacteria *P. syringae* pv. *maculicola* and after infection with the oomycete *P. infestans*, the causal agent of late blight disease. As shown in Fig. 3, *LOX1* and *DES* transcripts accumulated in parallel during infection of potato with *P. syringae* 6 and 12 hpi. In comparison, transcripts encoding

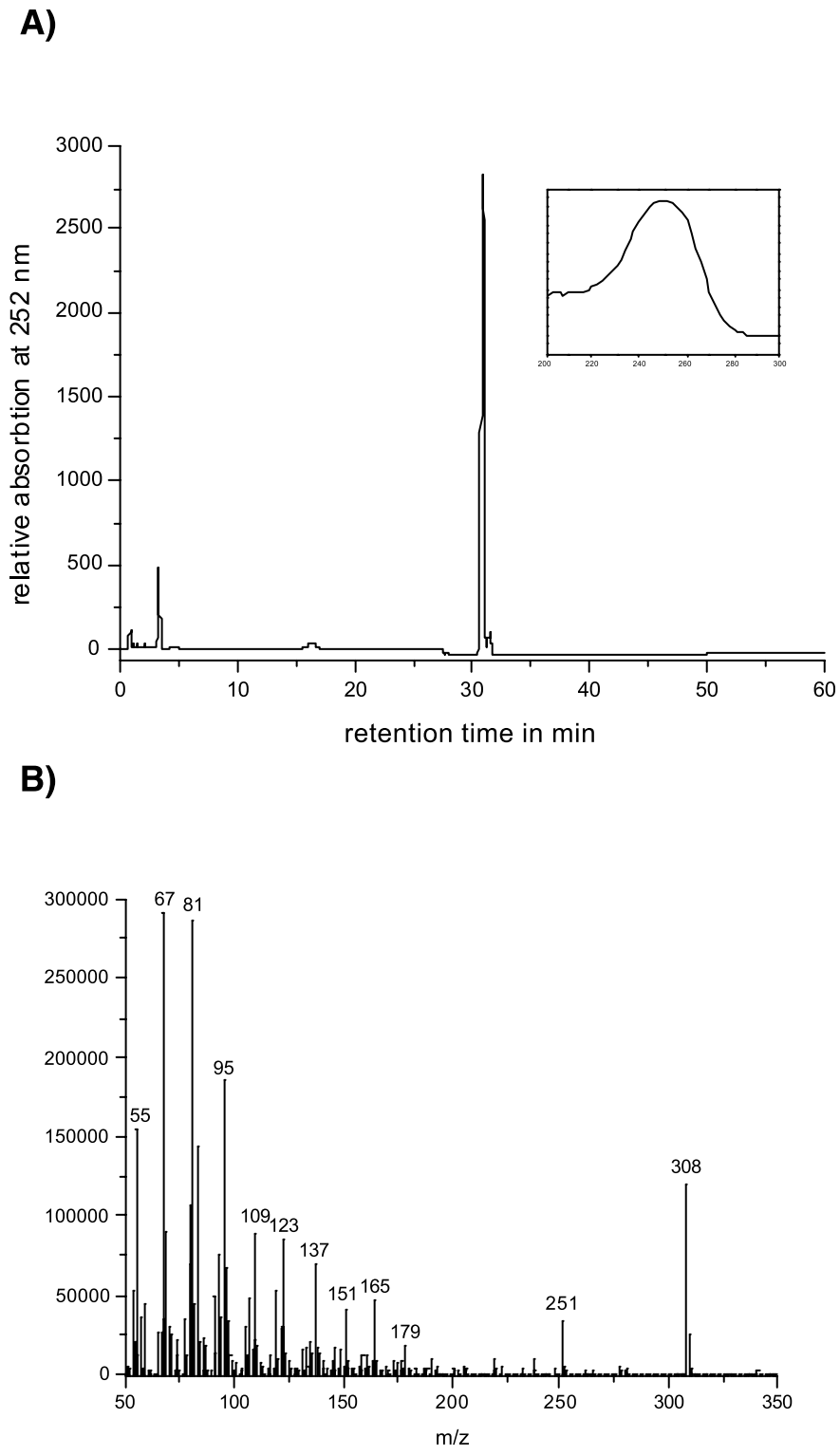


Fig. 2. HPLC analysis (A) of product formed by metabolism of 9-HPOD by recombinant StDES. Mass spectrum of the peak isolated from HPLC analysis analyzed by GC/MS (B). Recombinant DES from potato purified from *E. coli* cells expressing StDES was incubated with 9-HPOD at pH 6.5. A: The reaction product was analyzed as a free fatty acid derivative by HPLC-DAD and the chromatogram recorded at 252 nm is shown. The corresponding inset shows the UV spectrum of the product eluting at 31 min. B: The reaction product was analyzed as a methyl ester derivative by GC-MS. The mass spectrum of the product eluting at 18 min is shown.

4-CL, an enzyme of the phenylpropanoid pathway, started to accumulate earlier. In the compatible interaction of potato plants cv. Désirée with *P. infestans*, *LOX1* and *DES* transcripts increased 2 dpi, reaching maximal levels 3 and 4 dpi, similar to those of 4-CL (Fig. 4). These results indicate that the increases in CA and CnA levels in *P. infestans*-diseased potato leaves of 3 dpi [9] correlate with pathogen-induced activation of 9-LOX and DES genes.

Analysis of the organ-specific expression of StDES revealed that the corresponding mRNA was present in roots of green-

house-grown potato plants (data not shown) confirming the data obtained for tomato [8].

## 4. Discussion

Oxylipins are important signaling and defense compounds in eukaryotes whose synthesis may occur either enzymatically via the LOX pathway or by autoxidation [21]. The role of the LOX pathway in plant–pathogen interactions has been analyzed in numerous pathosystems [22] and the importance for 13-LOXs and their product jasmonate in resistance against insects and pathogens was demonstrated for *Arabidopsis thaliana* [23,24]. In contrast, in solanaceous plants, 9-LOXs appear to play a crucial role in defense responses [12], although it is not known which product of the 9-LOX pathway is necessary for the resistance response. The analysis of the oxylipin signature either in infected potato leaves [9] or in elicited potato cell suspension cultures [11] revealed the dominant induction of the DES pathway downstream of the 9-lipoxygenation of polyenoic fatty acids. Most recently, the missing piece, a cDNA coding for a DES, was isolated for the first time in a parallel research project from tomato [8]. However, in that report, the authors focussed on the identification and biochemical characterization of the recombinant protein. Here, we confirm the data found for the tomato DES, and, in addition, we present further evidence for an involvement of DES in plant–pathogen interactions by reporting on the isolation of a DES from elicitor-treated potato cells, expression of which is induced in potato plants by infection with either a bacterial pathogen and an oomycete pathogen, respectively.

By analyzing the cDNA of StDES at the amino acid level a high degree of identity could be observed in comparison to its isozyme from tomato. Moreover, by phylogenetic tree analysis, we found that both 9-DESs are closely related to 9-HPLs from cucumber and melon [20], indicating that, being younger than other isozymes of the *CYP74* family, they may have common ancestors. However, comparing the enzymatic properties of these more related isozymes sharing all the same substrate specificity against 9-hydroperoxy polyenoic fatty acid derivatives, an important difference becomes obvious. While all 9/13-HPLs and an 9/13-AOS described so far are more or less unspecific against the different positional isomers

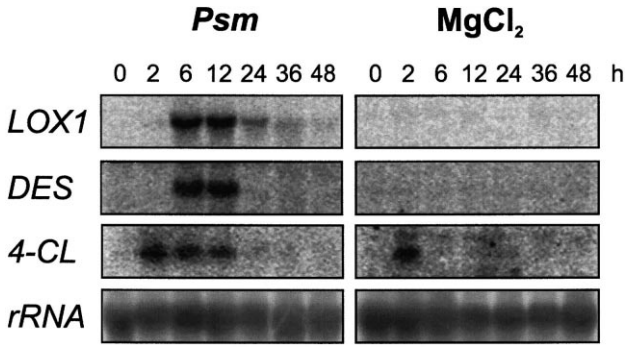


Fig. 3. Accumulation of DES transcripts in potato in response to infiltration of bacteria. RNA was isolated from potato leaves infiltrated with *P. syringae* pv. *maculicola* at the time points indicated (h), separated on formaldehyde gels, blotted onto nylon membranes and hybridized with radioactively labeled probes derived from the cDNA encoding LOX1, DES, 4-CL and, for standardization, with a radioactively labeled rRNA probe.

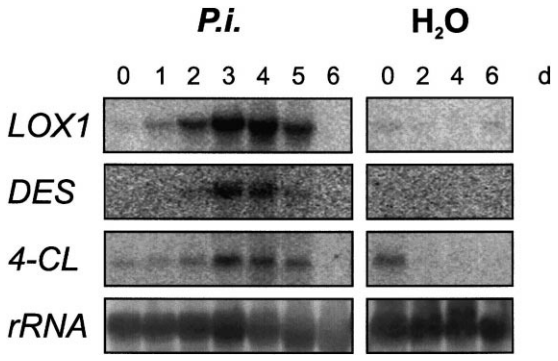


Fig. 4. Accumulation of DES transcripts in response to pathogen infection. RNA was isolated from potato leaves infected with *P. infestans* at the time points indicated (d), separated on formaldehyde gels, blotted onto nylon membranes and hybridized with radioactively labeled probes derived from the cDNA encoding LOX1, DES, 4-CL and, for standardization, with a radioactively labeled rRNA probe.

of hydroperoxy polyenoic fatty acids [20,25], the 9-DESs are the first enzymes from this subgroup that are rather specific against the 9-isomers. This had been described before only in case of 13-HPLs and 13-AOSs from tomato and *Arabidopsis* closely related to these enzymes [26–28]. This observation may give first hints towards the differences in the enzymatic mechanism and may be explained by structural differences in the active site of different members of the *CYP74* family.

The isolation of a cDNA clone encoding potato DES enabled us to study the expression of the corresponding genes in response to pathogen attack. Both after infection of potato with the bacterial pathogen *P. syringae* as well as in the disease-causing interaction with *P. infestans*, DES transcripts accumulate. Since this increase occurs in parallel to the pathogen-induced accumulation of 9-LOX mRNA, transcriptional activation of both 9-LOX and DES genes appear to be responsible for the higher levels of CA and CnA in elicitor-treated potato cells and *P. infestans*-diseased potato leaves [9,11]. This notion is further supported by the observation that the increase in 9-LOX and DES transcript levels (Fig. 4) precedes the accumulation of CA and CnA reported to occur in the compatible interaction of the potato cultivar Bintje and *P. infestans* [9]. The inhibitory effect of divinyl ethers on mycelial growth and spore germination of *P. infestans* [9] suggests a role of these compounds in defense against pathogens. Since CA and CnA accumulate earlier and to higher extents in resistant compared to susceptible potato cultivars in response to *P. infestans* infection [9], it will be of interest to analyze DES gene expression in potato cultivars with different degrees of resistance.

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