Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 65 (2004) 801–811
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

The allene oxide cyclase of barley (*Hordeum vulgare* L.)—cloning and organ-specific expression

Helmut Maucher[b], Irene Stenzel[a], Otto Miersch[a], Niels Stein[b], Manoj Prasad[b], Uwe Zierold[b], Patrick Schweizer[b], Conrad Dorer[a], Bettina Hause[c], Claus Wasternack[a,*]

[a]*Institute of Plant Biochemistry, Department of Natural Product Biotechnology, Weinberg 3, D-06120 Halle, Germany*
[b]*Institute of Plant Science and Crop Research, Corrensstrasse 3, D-06466 Gatersleben, Germany*
[c]*Institute of Plant Biochemistry, Department of Secondary Metabolism, Weinberg 3, D-06120 Halle/S., Germany*

Received 12 December 2003; received in revised form 12 January 2004

Abstract

The naturally occurring enantiomer of the various octadecanoids and jasmonates is established in a biosynthetic step catalyzed by the allene oxide cyclase (AOC). The AOC converts an allene oxide formed by an allene oxide synthase (AOS). Here, we show cloning and characterization of cDNAs encoding the AOC and a third AOS, respectively, in addition to the two AOSs previously published (Plant J. 21, 199–213, 2000). The ORF of the AOC-cDNA of 717 bp codes for a protein of 238 amino acid residues carrying a putative chloroplast target sequence. Overexpression without chloroplast target sequence revealed AOC activity. The *AOC* was found to be a single copy gene which mapped on chromosome 6H. AOC mRNA accumulation appeared in leaf segments upon treatment with various jasmonates, octadecanoids and ABA or during stress such as treatment with sorbitol or glucose solutions. Infection with powdery mildew activated *AOC* expression in susceptible and resistant lines of barley which correlated with *PR1b* expression. Among different tissues of barley seedlings, the scutellar node and leaf base accumulated AOC mRNA preferentially which correlated with accumulation of mRNAs for other biosynthetic enzymes (lipoxygenases, AOSs). AOC mRNA accumulation appeared also abundantly in parts of the root containing the tip and correlated with elevated levels of jasmonates. The data suggest a link of *AOC* expression and JA formation and support role of JA in stress responses and development of barley.

Keywords: Barley; Jasmonates; Allene oxide synthases; Allene oxide cyclase; Stress response; Tissue specificity; Powdery mildew

1. Introduction

During plant development or in adaptation to abiotic and biotic stresses, various signals can be generated from lipids. Among them are jasmonic acid (JA) and its precursor 12-**o**xo-**p**hyto**d**ienoic **a**cid (OPDA). They play an important role in developmentally or environmentally induced changes of gene expression. First hints were given by application experiments with jasmonates. Subsequently, endogenous rise of this group of compounds could be detected following environmental stress or in distinct developmental stages (Wasternack and Hause, 2002). This led to the question how biosynthesis of jasmonates is regulated.

The cyclopentanone compound JA and its derivatives, collectively named jasmonates, are formed from OPDA which is together with its derivatives collectively named octadecanoids. The initial reaction is the generation of ***α*-l**inol**e**nic **a**cid (α-LeA) from chloroplast lipids by phospholipase of the A1 type (PLA1), as evidenced by a recently identified JA-deficient mutant *dad1* (*d*elayed *a*nther *d*ehiscence*1*) (Ishiguro et al., 2001). α-LeA is the substrate of a 13-**l**ip**ox**ygenase (13-LOX), which specifically inserts molecular oxygen at carbon atom 13 leading to a hydroperoxide (***13****S*,9*Z*,11*E*,15*Z*)-**h**ydro**p**eroxy-(9,11,15)-**o**ctadeca**t**rienoic acid, 13-HPOT (Fig. 1). This

Abbreviations: ABA, abscisic acid; α-LeA, α-linolenic acid; AOC, allene oxide cyclase; AOS, allene oxide synthase; 13-HPOT, (13*S*,9*Z*,11*E*,15*Z*)-hydroperoxy-(9,11,15)-octadecatrienoic acid; JA, jasmonic acid; JA–L-Ile, JA–L-isoleucine conjugate; JAME, JA methyl ester; LOX, lipoxygenase; OPDA, 12-oxo-phytodienoic acid; OPDAME, OPDA methyl ester; OPR, OPDA reductase; PR1b, pathogenesis related protein 1b.

* Corresponding author. Tel.: +49(0)345-5582-1210; fax: +49(0)345-5582-1219.
E-mail address: cwastern@ipb-halle.de (C. Wasternack).

0031-9422/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2004.01.009

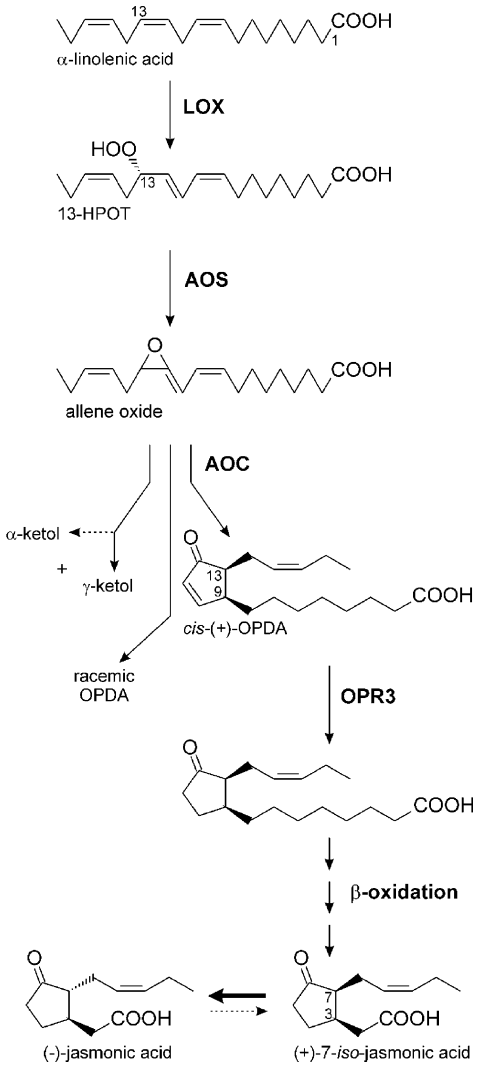

Fig. 1. Biosynthetic pathway of jasmonate biosynthesis.

reaction represents a part of the so-called LOX pathway in which at least seven different branches originate from 13-HPOT (Feussner et al., 2001). Although more than 50 different LOX forms have been cloned from various plants (Feussner and Wasternack, 2002), there is no final proof of a LOX form which acts specifically in JA biosynthesis. For barley three JA-inducible 13-LOX forms (*LOX2:Hv:1*, *LOX2:Hv:2*, *LOX2:Hv:3*) have been cloned (Bachmann et al., 2002; Vörös et al., 1998) and LOX protein was shown immunocytochemically to occur in barley mesophyll chloroplasts (Feussner et al., 1995).

In JA biosynthesis the 13-HPOT is dehydrated by the **a**llene **o**xide **s**ynthase (AOS) to an unstable allene oxide. AOSs are CYP450 enzymes. They have been cloned as single or multi-copy genes from various plant species and were grouped as the subfamily *CYP74A* (Feussner and Wasternack, 2002). To date two AOSs (*AOS1, AOS2*) were cloned from barley being active with 13-HPOT and 9-HPOT as substrates and were shown to be located in chloroplasts, even they lack a chloroplast target sequence (Maucher et al., 2000). The unstable allene oxide can hydrolyzes non-enzymatically into α- and γ-ketols or racemic OPDA. Under cellular conditions presumably most of the allene oxide is converted by an allene oxide cyclase (AOC), forming exclusively the *cis*-(+)-enantiomer (9*S*,13*S*) of OPDA. In the AOC-catalyzed step that enantiomeric structure is established which is present in the naturally occurring jasmonates. Therefore, the AOC is regarded to be of special importance in JA biosynthesis.

An AOC was purified from corn up to homogeneity (Ziegler et al., 1997). Subsequently, first cloning of an AOC was achieved from tomato (Ziegler et al., 2000). In tomato *AOC* occurs as a single copy gene located on chromosome 2 (Ziegler et al., 2000), whereas for *A. thaliana* four AOCs have been cloned and characterized which mapped on chromosome1 (*AOC4*) and chromosome 3 (*AOC1*, *AOC2*, *AOC3*) (Stenzel et al., 2003b). The tomato and *Arabidopsis* AOCs carry a putative chloroplast target sequence and were detected immunohistochemically within the chloroplast (Stenzel et al., 2003a,b; Ziegler et al., 2000).

The enantiomeric structure of *cis*-(+)-OPDA formed by the AOC *in vivo* and by the recombinant protein (Ziegler et al., 2000), is kept during reduction catalyzed by the **OP**DA **r**eductase (*OPR*). Interestingly, among three OPRs cloned from *A. thaliana* only OPR3 exhibits substrate specificity for *cis*-(+)-OPDA, and the JA-deficient mutants *dde1* and *opr1* are defective in OPR3 (Sanders et al., 2000; Stintzi and Browse, 2000). OPR3 of *A. thaliana* carries a peroxisomal target sequence (Stintzi and Browse, 2000) and is localized within the peroxisomes (Strassner et al., 2002), where the following three β-oxidation steps are assumed to occur. To date an enzyme which functions in β-oxidation of JA biosynthesis has not been characterized, and also cDNAs encoding such enzymes have not been isolated. Furthermore, to date it is unknown, how OPDA generated within the chloroplast is transferred into the peroxisomes.

In tomato, the AOC is specifically expressed in all vascular bundles and the surrounding parenchymatic cells (Hause et al., 2000), leading to preferential formation of JA at least in the main veins and to amplification of the wound response (Stenzel et al., 2003a). In contrast, in *A. thaliana* AOCs are constitutively expressed in all leaf tissues together with AOS and LOX.

The number of genes encoding enzymes specific for JA biosynthesis are different among various plant species. In tomato three AOSs, one AOC and three OPRs were detected (Howe et al., 2000; Sivasankar et al., 2000; Strassner et al., 2002; Ziegler et al., 2000). In *A. thaliana* one AOS, four AOCs and three OPRs were found (Laudert et al., 1996; Stenzel et al., 2003b; Strassner et al., 2002). This comparison suggests that JA

biosynthesis might be differentially regulated in these species. In case of AOS of *A. thaliana*, levels of mRNA, protein, activity as well as OPDA increased following treatment with salicylate (SA) (Laudert and Weiler, 1998). In barley, however, there was no effect of SA on AOS mRNA accumulation (Maucher et al., 2000). Furthermore, barley is different from dicotyledonous plants by its basal meristem carrying elevated levels of AOS and JA (Maucher et al., 2000) as well as by a lack of rise in JA upon wounding (unpublished data).

Due to these obvious differences between monocotyledonous and dicotyledonous plants in respect to JA biosynthesis and the importance of AOC mentioned above, we isolated and characterized an AOC cDNA from barley. Here, we show its characterization as well as the isolation of a third AOS cDNA from barley. The *AOC* is expressed upon treatment with jasmonates, octadecanoids and under different stress conditions including infection with powdery mildew. The expression of *AOC* correlates with that of three *AOSs* and the *LOX2:Hv:1*, the dominant LOX form among the three *13-LOXs* from barley. Moreover, the *AOC* expression appears preferentially in the leaf base, the scutellar node and root tip and correlates with elevated levels of JA.

2. Results

2.1. An AOC-encoding cDNA—cloning and mapping

In a sequencing program for ESTs from developing caryopses (3–29 days after pollination) an EST—designated HY01K08—was found to be highly homologous (53.8%) to a recently isolated cDNA from tomato coding for the AOC (Ziegler et al., 2000). The EST was found to be full length encompassing the entire AOC ORF which ranged from 62 to 778 bp, corresponding to a protein containing 238 amino acid residues. The calculated molecular mass was about 25.89 kDa. Sequence alignment indicates high homology to the tomato AOC, a rice AOC and a putative AOC from corn (Fig. 2). The barley AOC is rich in Ser residues at the N-terminus. Computer analysis of the first 100 amino acids was performed with the ChloroP V 1.1 program (http://www.dtu.dk/services/ChloroP) (Emanuelsson et al., 1999) and the TargetP program ver. 1.0 (http://www.cbs.dtu.dk/services/TargetP) (Emanuelsson et al., 2000). In both analyses a chloroplast localization was predicted. The putative cleavage site lies between amino acids 46 and 47. With the full length cDNA, a pI of 8.99 was calculated, whereas calculation without the putative chloroplast signal peptide revealed a pI of 5.58. Furthermore, homology appeared to two ESTs of barley found in the database. Sequencing of these ESTs, however, revealed a number of errors accumulating at the 5′ end region, typical for single path sequencing. Southern blot analysis and RFLP mapping indicate that *AOC* is a single copy gene in barley (Fig. 3).

Overexpression of a sequence in *E. coli* resulted under induction conditions in an additional band of 26 kDa which was absent in control bacteria transformed with the empty vector only (Fig. 4A). Analysis of extracts of bacteria containing only the empty vector revealed a 50% ratio of *cis*-(−)-OPDA and *cis*-(+)-OPDA formed indicating non-enzymatic OPDA formation (Fig. 4B). In extracts, however, of bacteria carrying the *AOC*-containing vector, exclusive formation of *cis*-(+)-OPDA was formed (Fig. 4C) which is indicative for AOC activity under the conditions used (Ziegler et al., 1997, 1999).

```
           1                                                                                                  90
AOC:Zm:1   MAAALRCPAS VR.VSGPA.A AGLAKVRQAS RVVAVSGARQ SRGGG...VA .VRASLFSPK .PAAAKDARP TKVQELYVYE INERDRESPA
AOC:Os:1   MAAAAPSRVS VR.AAAPGQT GGFAKIR..P QVVVAAAARS AGVSGRRARS .VRASLFSPK .PATPKDARP AKVQEMFVYE INERDRESPA
AOC:Hv:1   -MAVRPSSVS VR.AGASVS. ...AKLTPWR AARAGLGGRV SVSSGRRCGG PVRASLFSPK .PAVAMDARP SKVQELHVYE LNERDRESPA
AOC:Le:1   MATVSSASAA LRTISSSSSK LSSAFQTKKI QSFKLPNPLI SQNHKLTTTS TTASRSFSCK SQSTSTDSTN TEVQELSVYE INERDRGSPA
Consensus  ---------- -R-------- ---A------ ---------- ---------- ------FS-K ------D--- --VQE--VYE -NERDR-SPA

           91                                                                                                 180
AOC:Zm:1   YLRLSAKQTE NALGDLVPFT NKVYNGSLDK RLGVTAGICV LIQHVPDRNG DRYEAIYSFY FGDYGHISVQ GPYLTYEESY LAVTGGSGVF
AOC:Os:1   YLRLSAKQTE NALGDLVPFT NKLYSGSLDK RLGISAGICI LIQHVPERNG DRYEAIYSFY FGDYGHISVQ GPYLTYEESY LAVTGGSGVF
AOC:Hv:1   YLRLSANQSQ NALGDLVPFT NKVYNGSLDK RIGITAGICI LIQHVPERNG DRYEAIYSMY FGDYGHISLQ GPYLTYEESY LAVTGGSGVF
AOC:Le:1   YLRLSQK.TV NSLGDLVPFS NKLYTADLKK RIGITAGLCI LIKHEEEKKG DRYEAVYSFY FGDYGHIAVQ GAYLTYEETY LAVTGGSGIF
Consensus  YLRLS----- N-LGDLVPF- NK-Y---L-K R-GI-AG-CI LI-H-----G DRYEAIYS-Y FGDYGHI--Q G-YLTYEE-Y LAVTGGSGVF

           181                                                                  245
AOC:Zm:1   EGVYGQVKLN QIVFPFKIFY TFYLRGIPDL PRDLLCTPVP PSPTVEPTPA ARAAEPHASL DNYTN
AOC:Os:1   EGAYGQVKLN QIVFPFKIFY TFYLKGIPDL PRELLCTPVP PSPTVEPTPA AKATEPHACL NNFTN
AOC:Hv:1   EGVYGQVKLN QIVFPFKIFY TFYLKGIPDL PKELLCTPVP PSPTVEPTPA AKATEPHACL NNFTD
AOC:Le:1   AGVSGQVKLQ QLIFPFKLFY TFYLKGIPGL PSELLCTAVP PSPTVEPTPE AKACEEGAAL KNYTN
Consensus  -G--GQVKL- Q-VFPFK-FY TFYL-GIP-L P--LLCT-VP PSPTVEPTP- A-A-E--A-L -N-T-
```

Fig. 2. Amino acid sequence alignment of AOC from *Hordeum vulgare* cv. Salome (*AOC:Hv:1*, Acc.. No.AJ 308488), *Lycopersicon esculentum* (*AOC:Le:1*) (Ziegler et al., 2000), *Oryza sativa* (*AOC:Os:1*, Acc. No. AJ493664), and a putative AOC from *Zea mays* (*AOC:Zm:1*, Acc. No. AY103942).

2.2. A barley EST encoding a third AOS

In the Gatersleben barley EST project, one EST (Accession No. AL501477) was sequenced with significant homology to most known AOSs from monocotyledonous and dicotyledonous plants. The tblastx search showed highest homology with E-value of >3e-30 against the *Oryza sativa* genomic DNA. The *AOS1* and *AOS2* published for barley (Maucher et al., 2000) shared much lower similarity with E-value >9e-17. Consequently, we concluded the presence of an additional AOS (*AOS3*) in barley, and we isolated the corresponding BAC-clone from the barley BAC-library (Yu et al., 2000). After subcloning in a pBSKII- vector sequencing of the coding region of the putative *AOS3* was performed by primer walking. The genomic sequence contained no intron and one large open reading frame of 1524 bp giving a deduced polypeptide of 508 amino acids with a chloroplast target peptide predicted by the ChloroP V1.1 program. For analysis of AOS activity, we cloned several constructs corresponding to 507, 484, 479 and 439 amino acids and lacking the putative chloroplast target sequence in the bacterial vector pQE30. AOS activity was found for two constructs corresponding to 479 and 439 aa. The AOS3 was active only with 13-HPOT as substrate.

2.3. AOC mRNA accumulation in barley leaves upon treatments with jasmonates, octadecanoids and under different stress conditions

JA, OPDA and their derivatives are known to induce *AOC* gene expression in tomato and *Arabidopsis* (Stenzel et al., 2003a, b). In order to test AOC expression,

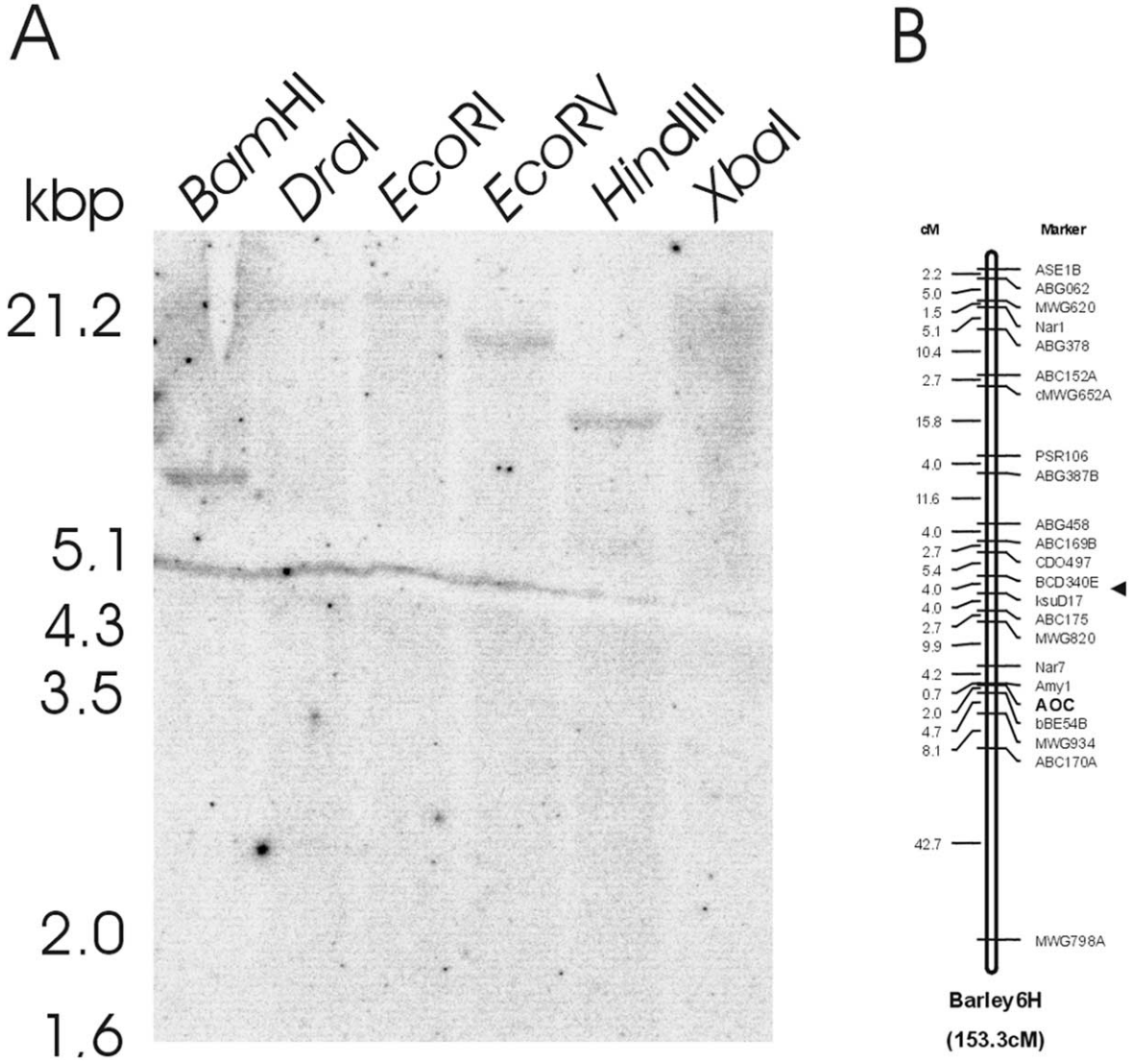

Fig. 3. Southern analysis of genomic barley DNA (A) and genetic mapping of *AOC* (B). Restriction with various enzymes revealed single AOC gene substantiated by one mapping position on chromosome 6H.

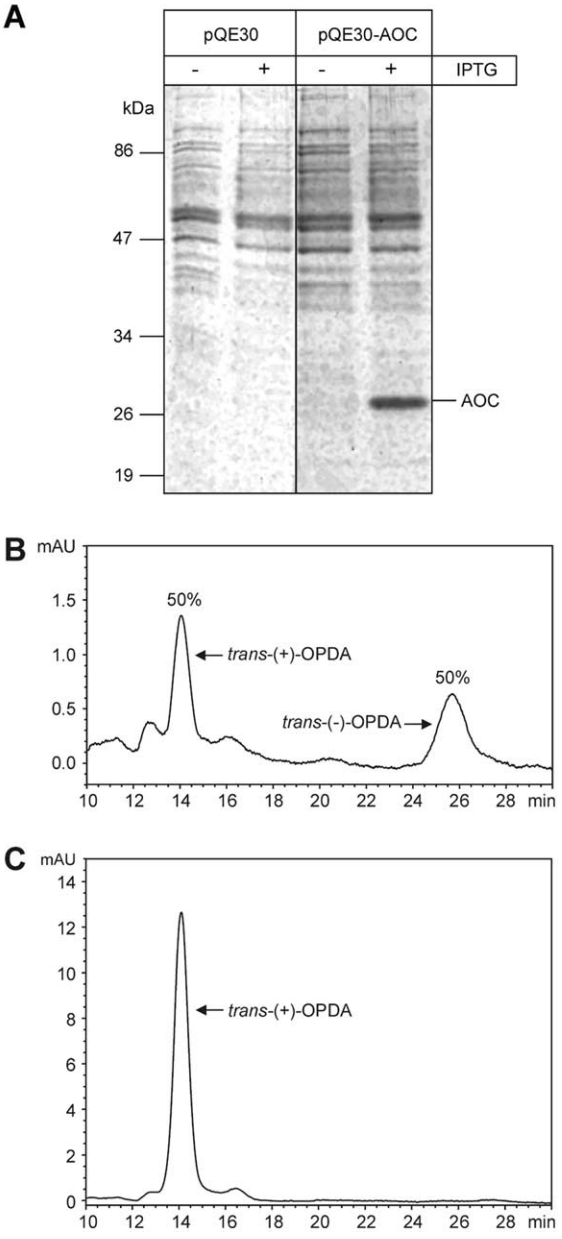

Fig. 4. Bacterial overexpression of barley AOC (A) and AOC activity assay (B, C). For (A) a truncated version of the cDNA clone *pHvAOC* as described in Experimental was subcloned into the vector pQE30 and transfected into the *E. coli* strain M15. Extracts from bacteria carrying the vector alone (pQE30) grown in the absence (−) or presence (+) of IPTG were separated by SDS-PAGE and stained with Coomassie blue. The position of abundantly appearing AOC is indicated. AOC activity tests were performed first with the empty vector (B) and led to a 50% ratio of *cis*-(−)-OPDA and *cis*-(+)-OPDA which was indicative for non-enzymatic OPDA formation. For chiral phase HPLC the corresponding *trans* forms were generated by alkaline conditions. In (C) the AOC activity test is shown for an extract of bacteria carrying the AOC construct. Exclusive formation of *cis*-(+)-OPDA, appearing as the corresponding *trans* form due to separation conditions, was indicative for AOC activity.

barley leaf segments were floated on water or 50 μM solutions of the various compounds or on 1 M sorbitol and 0.5 M glucose, respectively.

There was only a weak accumulation *AOC* mRNA at later times of floating on water, but all treatments with compounds and stress conditions revealed up-regulation of AOC gene expression (Fig. 5). Although mRNA levels did not peak sharply, AOC mRNA accumulation upon treatment with JA, JA–L-Ile, OPDA and OPDAME peaked at about 4 h contrasting with that of sorbitol and glucose treatment peaking after 24 h and 48 h, respectively. For JAME treatment a constant high level was detected after 4 h. The AOC mRNA accumulation in response to ABA appeared preferentially between 12 h and 24 h.

2.4. Simultaneous mRNA accumulation of LOXs, AOSs and AOC in different tissues of seedlings correlating with elevated levels of JA

Recently, preferential expression of two AOSs was shown to occur in the scutellar node and leaf base of barley seedlings correlating with elevated levels of JA (Maucher et al., 2000). A comparative analysis of cDNAs coding for three LOX forms, the third AOS and the AOC, both of them described here, revealed remarkable coincidence of the corresponding mRNA accumulation (Fig. 6A). The specificity of the LOX probes was described recently (Bachmann et al., 2002). Whereas the primary leaf exhibited only a residual *LOX2:Hv:1* mRNA level, significant accumulation of this mRNA was found in the scutellar node and the leaf base. *LOX2:Hv:2* mRNA and the *LOX2:Hv:3* mRNA was barely detectable and undetectable in these tissues, respectively. The specificity of the three AOS probes is

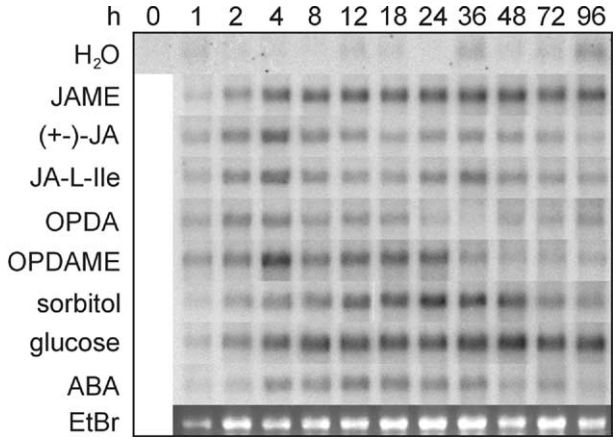

Fig. 5. AOC mRNA accumulation in barley leaf segments upon floating on water or solutions of various jasmonates and octadecanoids, respectively, (50 μM each) or on 1 M sorbitol, 0.5 M glucose and 50 μM ABA. Total RNA (20 μg per lane) was subjected to Northern blot analysis using ^{32}P-labeled insert of full-length AOC cDNA as described in Experimental. Loading was checked by ethidium bromide staining of RNA.

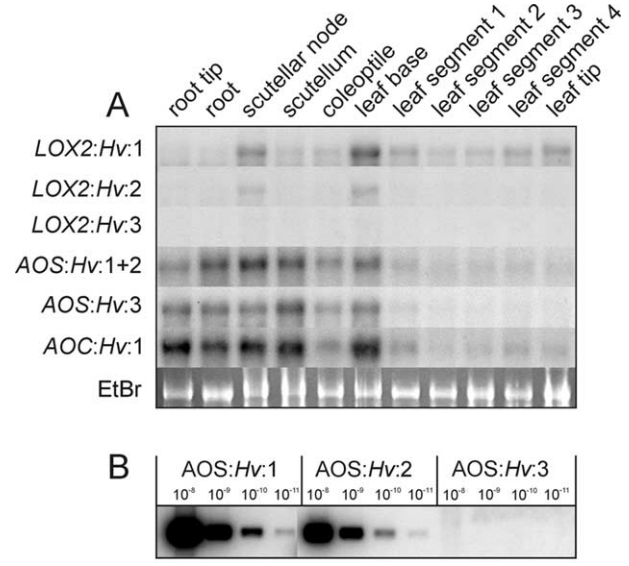

Fig. 6. mRNA accumulation of three LOX forms, three AOS forms and the AOC in different tissues of 7-days-old barley seedlings (A) and specificity test for each AOS probe (B). Total RNA (20 μg per lane) was subjected to Northern blot analysis as indicated in the legend of Fig. 4 and loading was checked by ethidium bromide staining of RNA. In case of AOS, specificity of each probe as well as the hybridization and washing conditions of each experiment were tested (B). A membrane was loaded with serial dilutions of all AOS-containing plasmids from 10^{-8} to 10^{-11} g. In the example shown, the filter was hybridized with the *AOS:Hv:1* probe, and cross-hybridizations was 60% for *AOS:Hv:2* and less than 0.1% for *AOS:Hv:3*, respectively. Consequently, in (A) AOS1 and AOS2 could not be distinguished. root*, first 10 mm of a root.

shown in Fig. 6B. The *AOS:Hv:1* probe cross-hybridized up to 60% with *AOS:Hv:2* but less than 0.1% with *AOS:Hv:3*. Consequently, gene specific mRNA accumulation can only be discussed for *AOS3*. Compared to the previously published mRNA accumulation of *AOS1/2* (Maucher et al., 2000) shown here for comparison, *AOS3* mRNA occurred with highly similar pattern. Furthermore, significant *AOS3* mRNA levels appeared in the tip-containing part of the root. Previously, we could show that elevated levels of JA in the scutellar node and the leaf base correlated with the accumulation of *AOS1/2* mRNA in these tissues (Maucher et al., 2000). Inspection of roots of 48 h and 72 h old seedlings by taking various segments up to the root tip revealed highest JA levels in the segment following the root tip (about 3 to 10 mm, Table 1).

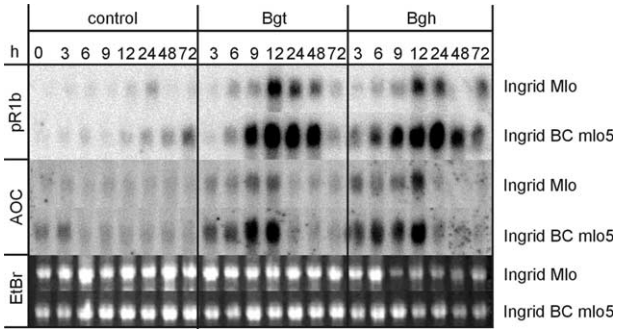

Fig. 7. AOC mRNA and PR1b mRNA accumulation in susceptible (Ingrid Mlo) and resistant (Ingrid BC mlo5) lines of barley following inoculation with barley powdery mildew (*Blumeria graminis* sp. *hordei*) or wheat powdery mildew (*Blumeria graminis* sp. *tritici*). Inoculation was performed with 56 and 47 spores per mm^2 on Ingrid Mlo and Ingrid BC mlo, respectively (*B. graminis* f. sp. *hordei*) and 75 and 94 spores per mm^2 (*B. graminis* f. sp. *tritici*). At indicated times total RNA was extracted and subjected to Northern blot analysis (10 μg per lane) using ethidium bromide staining as loading control. Hybridization was performed with ^{32}P labeled insert of the full length *AOC* cDNA and a *PR1b* probe.

2.5. AOC is expressed in barley near isogenic lines susceptible and resistant for powdery mildew

There is an ongoing discussion on the role of JA in the interaction of powdery mildew with barley (Hause et al., 1996; Schweizer, 1993). Therefore, we inspected *AOC* expression in a near-isogenic pair of susceptible (Ingrid Mlo) and resistant (Ingrid BC mlo5) barley lines following attack by the barley or the wheat powdery mildew fungus. For comparison, the mRNA of a marker gene for barley–pathogen interactions, *PR1b,* was also analyzed. As shown in Fig. 7, *PR1b* mRNA accumulation appeared to be stronger in the resistant line compared to the susceptible line, although it showed similar kinetics. Also *AOC* mRNA accumulated to higher levels in the resistant line compared to the susceptible line. Interestingly, the *AOC* mRNA accumulation preceded that of PR1b, and was more transient. For both, PR1 and AOC, no difference in mRNA accumulation could be observed between attack with barley or wheat powdery mildew.

Table 1
Jasmonate content (in pmoles per g fresh weight) in root segments of barley seedlings. Seeds were imbibed on wet filter paper for the indicated times. Segments were collected from at least 80 roots and processed for JA determination. Three independent extractions and analyses were performed giving similar values that varied by about 15%. One series of data is given.

	Root Tip	Segment I	Segment II	Segment III (next to the shoot)
Length of segments	3 mm	7 mm	10 mm	18 mm
48 h after imbibition	1028	2370		497
72 h after imbibition	1611	2800	982	527

3. Discussion

The first jasmonate-induced alteration of gene expression was observed with barley leaf segments (Weidhase et al., 1987b). As in other plants, the JA-induced proteins of barley belong to various classes, reflecting the pleiotropic function of JA. A common feature, however, of all plant species analyzed to date is their ability to respond to biotic or abiotic stresses with an endogenous rise of JA, which appears within the first few hours upon the onset of stress (Wasternack and Hause, 2002). In barley, stress-induced increase of JA and related compounds is relatively late, occurs first transiently and is followed by a steady increase (Kramell et al., 2000).

As for other plant species, isolation of cDNAs coding for enzymes of JA biosynthesis is the main tool to analyze this rise in JA in barley. In case of LOXs, however, it is still unclear which of the three 13-LOXs identified are active in JA biosynthesis (Bachmann et al., 2002). In addition to the previously described AOS1 and AOS2 (Maucher et al., 2000), here we characterized a third AOS exhibiting a similar expression pattern in different tissues of seedlings as the AOS1/2 (Fig. 6). Based on sequence comparison, the AOS3 might be a 13-AOS, which was confirmed by the conversion of only 13-HPOT. Sequence comparison for AOS1 and AOS2 showed that they form a new group in the phylogenetic tree, the 9/13-AOSs (Feussner and Wasternack, 2002), which is also indicated by the ability of AOS1 to convert 9-hydroxy and 13-hydroxy polyunsaturated fatty acids (Maucher et al., 2000). In tomato, a 9-AOS was identified recently being expressed preferentially in roots (Itoh et al., 2002). This positional specificity among the different AOSs, also known for the various LOX forms in many species (Feussner and Wasternack, 2002), would allow the plant to build up two separate groups of compounds derived either from 9-LOX products or from 13-LOX products. Formation of 9-LOX products was preferentially found in response to pathogen attack (Göbel et al., 2002). It will be interesting to see how such a specificity in oxylipin metabolism is kept in the AOS-catalyzed step.

Following treatments with jasmonates or octadecanoids, barley leaves accumulated *AOC* mRNA significantly in the first 4 h of treatment, suggesting a positive feedback in JA biosynthesis. Such a positive feedback loop in JA biosynthesis has been repeatedly deduced from expression data as for rice (Agrawal et al., 2003) or *Arabidopsis* (Sasaki et al., 2001; Stenzel et al., 2003b). However, such a mRNA accumulation following various treatments was not necessarily related to the early rise in JA: (i) Upon sorbitol treatment of barley leaves, *AOC* mRNA accumulation peaked at about 24 h, whereas the first rise in JA appears between 5 and 8 h (Kramell et al., 2000), (ii) despite transcriptional activation of JA biosynthetic genes in barley leaves, there is no dilution of applied deuterated JA within 24 h (Kramell et al., 2000), and (iii) overexpression of *AOS* or *AOC* in *Arabidopsis*, tobacco or tomato did not lead to constitutively elevated levels of JA in leaves (Laudert et al., 2000; Stenzel et al., 2003a; Wang et al., 1999). Substrate generation by wounding of leaves of these plants, however, leads to increased rise in JA levels. This accords to the situation in non-transgenic *Arabidopsis* plants. Here, LOX, AOS and AOCs occur in leaves abundantly but are active in JA biosynthesis only upon substrate generation by wounding or other stimuli (Stenzel et al., 2003b). It is tempting to speculate that the early rise in JA level detected for many plants for the first hour of any treatment, may attribute to the positive feed back loop seen as accumulation of mRNAs of JA biosynthetic enzymes such as AOC or AOS.

Interestingly, the *AOC* of barley, characterized here exhibited similar expression pattern than the *AOS*s (Fig. 6) (Maucher et al., 2000). This is in contrast to numerous expression patterns recorded for *AOC* and *AOS* of rice, where AOC mRNA accumulation differed in time and strength from *AOS* mRNA accumulation (Agrawal et al., 2002, 2003). In contrast to leaves stressed or treated with jasmonates (Fig. 5) (Kramell et al., 2000) tissues of a barley seedling exhibited a strict correlation of JA content and transcript accumulation of *AOSs* and *AOC* in different organs (Fig. 6A) (Maucher et al., 2000). Tissues such as the scutellar node and the leaf base accumulate *LOX*, *AOS* and *AOC* mRNAs significantly and carry up to 20-fold higher JA content than the leaf blade (Maucher et al., 2000). Also tip-containing parts of a root exhibited strong AOC mRNA accumulation, and the tissue directly following the tip showed up to 6-fold higher JA level than root tissues near the shoot. Possibly, JA biosynthesis is self-activated during seedling development by expression of JA biosynthetic genes. JA formation in turn may lead to expression of other JA-inducible genes known to occur during barley seedling development (Hause et al., 1996). Interestingly, one of the genes encoding the JA-inducible protein of 23 kDa (JIP23) of barley leaves, is specifically expressed in tissues with high expression of *AOS*s and *AOC* (Fig. 6A), (Hause et al., 1996, 1999a; Maucher et al., 2000). Since the JA levels were also elevated in these tissues, *JIP23* expression seems to be the consequence of these higher JA levels. Such a scenario of JA biosynthetic capacity, and JA responsive gene expression was also observed in arbuscular mycorrhizal barley roots (Hause et al., 2002). Interestingly, in tomato flowers, the abundant occurrence of AOC was accompanied with elevated JA and OPDA levels in pistils, and constitutive overexpression of *AOC* increased several-fold levels of most jasmonates and octadecanoids (Miersch et al., unpublished). Taken together, data presented here for barley support that regulation of JA biosynthesis may differ between developing organs and stressed leaves.

Jasmonates are signals, together with ethylene, in the interaction of plants with necrotrophic pathogens lead-

ing in case of *Arabidopsis* to defensin/thionin expression or the establishment of induced systemic resistance (ISR) (Pieterse et al., 2002; Thomma et al., 1998; Van Wees et al., 1999). Also in the basal resistance of plants against necrotrophic pathogens JA may play a role (Ton et al., 1999). Moreover, defense responses in plants against biotrophic pathogens like powdery mildews appear to be more dependent on salicylic acid (Metraux et al., 2002), but can be linked to jasmonate by cross-talk via NPR1 (Spoel et al., 2003). However, in *Blumeria*-attacked barley leaves neither evidence for a signaling role of salicylic acid nor of JA has been obtained so far (Hückelhoven et al., 1999; Kogel et al., 1995; Schweizer et al., 1993; Vallélian-Bindschedler et al., 1998). Recently, induction of systemic resistance of barley against *Blumeria graminis* has been reported by localized application of JAME (Walters et al., 2002) suggesting a role of JA signaling in protecting the developing younger part of growing plants rather than in direct defense of attacked plant tissue. A systemic but not a local resistance induction by JA has also been observed in rice (Schweizer et al., 1998) and a JA-inducible LOX of barley was also induced by activators of systemic acquired resistance but not by pathogens (Hause et al., 1999a, b). Using the AOC cloned from barley as marker for JA biosynthesis, we re-investigated the role of JA in powdery mildew-attacked barley. In both susceptible as well as in *mlo5*-resistant and non-host-resistant interactions, AOC transcript levels increased. It is interesting to note that there exist at least two genes in barley with homology to OPRs that are both induced by *Blumeria graminis* (P. Schweizer, unpublished data). These gene expression data appear to contrast with previous results on a lack of JA accumulation in *Blumeria*-attacked barley (Kogel et al., 1995). It might be that localized accumulation of JA in specific cells or cell types escaped detection that was based on whole-leaf extracts. However, taking advantage from a immunocytological detection of a jasmonate-induced protein as a reporter for JA-levels, such a local rise in JA following attack of barley leaves by *Blumeria* could not be found (Hause et al., 1997). Alternatively, there might be enhanced JA turnover in *Blumeria*-attacked leaves, requiring enhanced biosynthetic capacity in order to maintain "resting" levels of JA. Reverse-genetic approaches will be required to help answering the question of the biological relevance of the oxylipin pathway in *Blumeria*-attacked barley.

4. Experimental

4.1. Plant material and treatments

Seedlings of barley (*Hordeum vulgare* cv. Salome) were grown under greenhouse conditions at 25 °C and 70% relative humidity with 16 h light (minimum intensity of 130 μmol m^{-2} s^{-1}) for seven days. For treatments, primary leaves were cut into 5 cm segments starting 1 cm below the tip and were floated on water or on aqueous solution of 50 μM JA methyl ester (JAME), 50 μM (±)-JA, 50 μM JA–L-isoleucine conjugate (JA–L-Ile), 50 μM OPDA, 50 μM OPDAME, 1 M sorbitol, 0.5 M glucose or 50 μM (±)-ABA. Floating of leaf segments was performed in Petri dishes containing 25 ml solution per six segments at 25 °C under continuous light (120 μmol·m^{-2} s^{-1}) provided by fluorescent lamps (NARVA Berlin, NC, USA, 250/01). For JA determination of roots, seeds were imbibed on wet filter paper in the dark at 25 °C for indicated times.

For inoculation experiments with the barley powdery mildew fungus *Blumeria graminis* f. sp. *hordei* (Bgh) or with wheat powdery mildew *Blumeria graminis* f. sp. *tritici* (Bgt), near isogenic (BC7) barley plants cv Ingrid and Ingrid BC *mlo*5 were grown in the greenhouse at 18 °C (night) to 22 °C (day). Daylight was supplemented with metal halogen lamps in the morning and in the evening, resulting in a photoperiod of 16 h. Seven-day-old seedlings were used for inoculation in a settling tower by shaking heavily Bgh- or Bgt-infected plants over the test plants, resulting in an inoculum density on leaves of 47–56 conidia mm^{-2} (Bgh) or 75–94 conidia mm^{-2} (Bgt). Control and inoculated plants were incubated next to each other and exposed to indirect daylight (11 h day^{-1}) at a constant temperature of 20 °C. At the times indicated, primary leaf material was frozen in liquid N_2 and used for RNA extraction.

Blumeria graminis f. sp. *hordei*, strain 4.8 carrying AvrMla9, was cultivated by weekly inoculation of 7-day-old seedlings of barley cv Golden Promise. *Blumeria graminis* f. sp. *tritici*, strain FAL92315 carrying no known avirulence, was cultivated by weekly inoculation of 7-day-old seedlings of wheat cv. Kanzler. Seven days post inoculation, conidia were used for inoculating test plants by shaking inoculated plants over test plants in the settling tower.

4.2. Isolation of AOC cDNA and expression in Escherichia coli

The EST HY01K08 of the barley genome program at the IPK Gatersleben (Germany) was used for overexpression. For that a sequence corresponding to aa 60 to aa 238, thus lacking the putative chloroplast target sequence was subcloned into pQE30. PQE30 with or without insert was transformed into the host strain *E. coli* M15. Total protein of isopropyl-β-thiogalactopyranoside (IPTG)-induced or non induced cultures was isolated and purified as described (Maucher et al., 2000). The resulting supernatants were used for AOC activity tests as described (Stenzel et al., 2003b;

Ziegler et al., 2000). Corresponding overexpression was performed with *AOS*-containing sequences lacking the putative chloroplast target sequence and AOS activity was analyzed as described (Maucher et al., 2000).

4.3. Extraction of RNA, Northern blot analysis and Southern blot analysis

Total RNA was extracted from frozen tissues as described (Stenzel et al., 2003a) except for infection experiments, where RNA was extracted according to (Vallélian-Bindschedler et al., 1998). After ethanol precipitations 10 and 20 μg, respectively, per lane was subjected to RNA gel blot electrophoresis according to Sambrook et al. (1989), and gel loading was checked by comparing ethidium bromide-stained rRNAs. Hybridization was performed at 60 °C for 16 h with ^{32}P-labelled full-length cDNA of AOC. Gene-specific probes of LOXs were used as described by (Bachmann et al., 2002). Gene-specific probes of AOSs were used as described in the legend of Fig. 6B.

4.4. Southern analysis and mapping

To map the AOC gene, one hundred and fifty double haploid (DHs) lines derived from the cross of Steptoe×Morex (SM) (Kleinhofs et al., 1993) was used in the present study.

DNA extraction and Southern analysis were carried out as described earlier (Graner et al., 1991). A set of six restriction enzymes (*Bam*HI, *Hind*III, *Eco*RI, *Eco*RV, *Xba*I and *Dra*I) was used to digest genomic DNA. Autoradiography was performed by exposure of hybridized blots to imaging plates (Fuji Photo Film, Japan) and subsequent signal detection on a phosphoimager (FLA-3000, Fuji, Japan).

The markers were mapped using MAPMAKER v. 2.0 program (Lander et al., 1987) based on Kosambi's mapping function (Kosambi, 1944) and using a minimum LOD score of 3.0 and maximum recombination frequency of 50%. We used a 223-point SM base map (http://gnome.agrenv.mcgill.ca/info/smmap.htm) as a reference map to map the AOC gene.

4.5. Extraction and quantification of jasmonates and octadecanoids

Tissues (0.5 g f.w.) were frozen in liquid nitrogen, homogenized in a mortar and extracted with 5 ml 80% (v/v) methanol. For quantification of JA and JAME ($^2H_6$)-JA was added in an appropriate amount before extraction. Upon ion exchange chromatography on DEAE Sephadex A-25 cartridges, RP-HPLC and GC-MS/SIM analyses were performed as described (Hause et al., 2000; Kramell et al., 2000).

Acknowledgements

We thanks B. Ortel for technical assistance and C. Dietel for typing the manuscript. Financial support was given by the Deutsche Forschungsgemeinschaft (SFB 363, project C5) to C.W. and O.M. and the Fonds der Chemischen Industrie to C.W.

References

Agrawal, G.K., Rakwal, R., Jwa, N.-S., Han, K.-S., Agrawal, V.P., 2002. Molecular cloning and mRNA expression analysis of the first rice jamonate biosynthetic pathway gene allene oxide synthase. Plant Physiol. Biochem. 40, 771–782.

Agrawal, G.K., Jwa, N.-S., Agrawal, S.K., Tamogami, S., Iwahashi, H., Rakwal, R., 2003. Cloning of novel rice allene oxide cyclase (OsAOC): mRNA expression and comparative analysis with allene oxide synthase (OsAOS) gene provides insight into the transcriptional regulation of octadecanoid pathway biosynthetic genes in rice. Plant Sci. 164, 979–992.

Bachmann, A., Hause, B., Maucher, H., Garbe, E., Vörös, K., Weichert, H., Wasternack, C., Feussner, I., 2002. Jasmonate-induced lipid peroxidation in barley leaves initiated by distinct 13-LOX forms of the chloroplast. Biol. Chem. 383, 1645–1657.

Emanuelsson, O., Nielsen, H., von Heijne, G., 1999. ChloroP, a neural network-based method for predicting chloroplast transit peptides and their cleavage sites. Protein Science 8, 978–984.

Emanuelsson, O., Nielsen, H., Bonnak, S., von Heijne, G., 2000. Predicting subcellular localization of proteins based on N-terminal amino acid sequence. J. Mol. Biol. 300, 1005–1016.

Feussner, I., Wasternack, C., 2002. The lipoxygenase pathway. Annu. Rev. Plant Biol. 53, 275–297.

Feussner, I., Kühn, H., Wasternack, C., 2001. Lipoxygenase dependent degradation of storage lipids. Trends Plant Sci. 6, 368–373.

Feussner, I., Hause, B., Vörös, K., Parthier, B., Wasternack, C., 1995. Jasmonate-induced lipoxygenase forms are localized in chloroplasts of barley leaves (*Hordeum vulgare* cv. Salome). Plant J. 7, 949–957.

Göbel, C., Feussner, I., Hamberg, M., Rosahl, S., 2002. Oxylipin profiling in pathogen-infected potato leaves. Biochim. Biophys. Acta 1584, 55–64.

Graner, A., Jahoor, A., Schondelmaier, H., Siedler, K., Pillen, K., Wenzel, G., Herrmann, R.G., 1991. Construction of an RFLP map of barley. Theor. Appl. Genet. 83, 250–256.

Hause, B., Kogel, K.-H., Parthier, B., Wasternack, C., 1997. In barley leaf cells, jasmonates do not act as a signal during compatible or incompatible interactions with the powdery mildew fungus (*Erysiphe graminis* f. sp. *hordei*). J. Plant Physiol. 150, 127–132.

Hause, B., Hertel, S.C., Klaus, D., Wasternack, C., 1999a. Cultivar-specific expression of the jasmonate-induced protein of 23 kDa (JIP-23) occurs in *Hordeum vulgare* L. by jasmonates but not during seed germination. Plant Biol. 1, 83–89.

Hause, B., Demus, U., Teichmann, C., Parthier, B., Wasternack, C., 1996. Developmental and tissue-specific expression of JIP-23, a jasmonate-inducible protein of barley. Plant Cell Physiol. 37, 641–649.

Hause, B., Vörös, K., Kogel, K.-H., Besser, K., Wasternack, C., 1999b. A jasmonate-responsive lipoxygenase of barley leaves is induced by plant activators but not by pathogens. J. Plant Physiol. 154, 459–462.

Hause, B., Maier, W., Miersch, O., Kramell, R., Strack, D., 2002. Induction of jasmonate biosynthesis in arbuscular mycorrhizal barley roots. Plant Physiol. 130, 1213–1220.

Hause, B., Stenzel, I., Miersch, O., Maucher, H., Kramell, R., Ziegler, J., Wasternack, C., 2000. Tissue-specific oxylipin signature of tomato flowers: allene oxide cyclase is highly expressed in distinct flower organs and vascular bundles. Plant J. 24, 113–126.

Howe, G.A., Lee, G.I., Itoh, A., Li, L., DeRocher, A.E., 2000. Cytochrome P450-dependent metabolism of oxylipins in tomato. Cloning and expression of allene oxide synthase and fatty acid hydroperoxide lyase. Plant Physiol. 123, 711–724.

Hückelhoven, R., Fodor, J., Preis, C., Kogel, K.H., 1999. Hypersensitive cell death and papilla formation in barley attacked by the powdery mildew fungus are associated with hydrogen peroxide but not with salicylic acid accumulation. Plant Physiol. 119, 1251–1260.

Ishiguro, S., Kawai-Oda, A., Ueda, J., Nishida, I., Okada, K., 2001. The defective in anther dehiscence 1 gene encodes a novel phospholipase A1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in *Arabidopsis*. Plant Cell 13, 2191–2209.

Itoh, A., Schilmiller, A., McCaig, B.C., Howe, G.A., 2002. Identification of a jasmonate-regulated allene oxide synthase that metabolizes 9-hydroperoxides of linoleic and linolenic acids. J. Biol. Chem. 277, 46051–46658.

Kleinhofs, A., Kilian, A., Mas, M.R.M.B., Chen, H.P., Lapitan, N., Fenwick, A.M., Heun, M., Franckowiak, J.D., Hoffman, D., Skadsen, R., Steffenson, B.J., 1993. A molecular, isozyme and morphological map of the barley (*Hordeum vulgare*) genome. Theor. Appl. Genet. 86, 705–712.

Kogel, K.H., Ortel, B., Jarosch, B., Atzorn, R., Schiffer, R., Wasternack, C., 1995. Resistance in barley against the powdery mildew fungus (*Erysiphe graminis* f. sp. hordei) is not associated with enhanced levels of endogenous jasmonates. Eur. J. Plant Pathol. 101, 319–332.

Kosambi, D.D., 1944. The estimation of map distances from recombination values. Annu. Eugen. 12, 172–175.

Kramell, R., Miersch, O., Atzorn, R., Parthier, B., Wasternack, C., 2000. Octadecanoid-derived alteration of gene expression and the 'oxylipin signature' in stressed barley leaves-implications for different signalling pathways. Plant Physiol. 123, 177–186.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., Newburg, L., 1987. MAPMAKER: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1, 174–181.

Laudert, D., Schaller, A., Weiler, E.W., 2000. Transgenic *Nicotiana tabacum* and *Arabidopsis thaliana* plants overexpressing allene oxide synthase. Planta 211, 163–165.

Laudert, D., Pfannschmidt, U., Lottspeich, F., Holländer-Czytko, H., Weiler, E.W., 1996. Cloning, molecular and functional characterization of *Arabidopsis thaliana* allene oxide synthase (CYP74), the first enzyme of the octadecanoid pathway to jasmonates. Plant Mol. Biol. 31, 323–335.

Laudert, D., Weiler, E.W., 1998. Allene oxide synthase: a major control point in *Arabidopsis thaliana* octadecanoid signalling. Plant J. 15, 675–684.

Maucher, H., Hause, B., Feussner, I., Ziegler, J., Wasternack, C., 2000. Allene oxide synthases of barley (*Hordeum vulgare* cv. Salome)-tissue specific regulation in seedling development. Plant J. 21, 199–213.

Metraux, J.P., Nawrath, C., Genoud, T., 2002. Systemic acquired resistance. Euphytica 124, 237–243.

Pieterse, C.M.J., Van Wees, S.C.M., Ton, J., Van Pelt, J.A., Van Loon, L.C., 2002. Signalling in rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. Plant Biol. 4, 535–544.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York.

Sanders, P.M., Lee, P.Y., Biesgen, C., Boone, J.D., Beals, T.P., Weiler, E.W., Goldberg, R.B., 2000. The Arabidopsis *DELAYED DEHISCENCE1* gene encodes an enzyme in the jasmonic acid synthesis pathway. Plant Cell 12, 1041–1061.

Sasaki, Y., Asamizu, E., Shibata, D., Nakamura, Y., Kaneko, T., Awai, K., Amagai, M., Kuwata, C., Tsugane, T., Masuda, T., Shimada, H., Takamiya, K., Ohta, H., Tabata, S., 2001. Monitoring of methyl jasmonate-responsive gene in Arabidopsis by cDNA macroarray: self-activation of jasmonic acid biosynthesis and crosstalk with other phytohormone signaling pathways. DNA Res. 8, 153–161.

Schweizer, P., Gees, R., Mosinger, E., 1993. Effect of jasmonic acid on the interaction of barley *Hordeum vulgare* L. with the powdery mildew *Erysiphe graminis* f sp. *hordei*. Plant Physiol. 102, 503–511.

Schweizer, P., Buchala, A., Dudler, R., Metraux, J.P., 1998. Induced systemic resistance in wounded rice plants. Plant J. 14, 475–481.

Sivasankar, S., Sheldrick, B., Rothstein, S.J., 2000. Expression of allene oxide synthase determines defense gene activation in tomato. Plant Physiol. 122, 1335–1342.

Spoel, S.H., Koornneef, A., Claessens, S.M.C., Korzelius, J.P., Van Pelt, J.A., Mueller, M.J., Buchala, A.J., Métraux, J.-P., Brown, R., Kazan, K., Van Loon, L.C., Dong, X., Pieterse, C.M.J., 2003. NPR1 modulates cross-talk between salicylate- and jasmonate-dependent defense pathways through a novel function in the cytosol. Plant Cell 15, 750–770.

Stenzel, I., Hause, B., Maucher, M., Pitzschke, A., Miersch, O., Ziegler, J., Ryan, C.A., Wasternack, C., 2003a. Allene oxide cyclase dependence of the wound response and vascular bundle specific generation of jasmonate in tomato- amplification in wound-signalling. Plant J. 33, 577–589.

Stenzel, I., Hause, B., Miersch, O., Kurz, T., Maucher, H., Weichert, H., Ziegler, J., Feussner, I., Wasternack, C., 2003b. Jasmonate biosynthesis and the allene oxide cyclase family of *Arabidopsis thaliana*. Plant Mol. Biol. 51, 895–911.

Stintzi, A., Browse, J., 2000. The *Arabidopsis* male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. Proc. Natl. Acad. Sci. U.S.A. 97, 10625–10630.

Strassner, J., Schaller, F., Frick, U.B., Howe, G.A., Weiler, E.W., Amrhein, N., Macheroux, P., Schaller, A., 2002. Characterization and cDNA-microarray expression analysis of 12-oxophytodienoate reductases reveals differential roles for octadecanoid biosynthesis in the local versus the systemic wound response. Plant J. 32, 585–601.

Thomma, B.P.H.J., Eggermont, K., Penninckx, I.A.M.A., Mauch-Mani, B., Vogelsang, R., Cammue, B.P.A., Broekaert, W.F., 1998. Separate jasmonate-dependent and salicylate-dependent defense-response pathways in Arabidopsis are essential for resistance to distinct microbial pathogens. Proc. Natl. Acad. Sci. U.S.A. 95, 15107–15111.

Ton, J., Pieterse, C.M., Van Loon, L.C., 1999. Identification of a locus in *Arabidopsis* controlling both the expression of rhizobacteria-mediated induced systemic resistance (ISR) and basal resistance against *Pseudomonas syringae* pv. tomato. Mol. Plant Microbe Interact. 12, 911–918.

Vallélian-Bindschedler, L., Metraux, J.-P., Schweizer, P., 1998. Salicylic acid accumulation in barley is pathogen specific but not required for defense-gene activation. Mol. Plant Microbe Interact. 11, 702–705.

Van Wees, S.C.M., Luijendijk, M., Smoorenburg, I., van Loon, L.C., Pieterse, M.J., 1999. Rhizobacteria-mediated induced systemic resistance (ISR) in Arabidopsis is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene *Atvsp* upon challenge. Plant Mol. Biol. 41, 537–549.

Vörös, K., Feussner, I., Kühn, H., Lee, J., Graner, A., Löbler, M., Parthier, B., Wasternack, C., 1998. Characterization of methyljasmonate-inducible lipoxygenase from barley (*Hordeum vulgare* cv. Salome) leaves. Eur. J. Biochem. 251, 36–44.

Walters, D., Cowley, T., Mitchell, A., 2002. Methyl jasmonate alters polyamine metabolism and induces systemic protection against powdery mildew infection in barley seedlings. J. Exp. Bot. 53, 747–756.

Wang, C., Avdiushko, S., Hildebrand, D.F., 1999. Overexpression of a cytoplasm-localized allene oxide synthase promotes the wound-induced accumulation of jasmonic acid in transgenic tobacco. Plant Mol. Biol. 40, 783–793.

Wasternack, C., Hause, B., 2002. Jasmonates and octadecanoids:

Signals in plant stress responses and plant development. Progr. Nucleic Acid Res. Mol. Biol. 72, 165–221.

Weidhase, R.A., Kramell, R., Lehmann, J., Liebisch, H.W., Lerbs, W., Parthier, B., 1987b. Methyl jasmonate-induced changes in the polypeptide pattern of senescing barley leaf segments. Plant Sci. 51, 177–186.

Yu, Y., Tomkins, J., Waugh, R., Frisch, D., Kudrna, D., Kleinhofs, A., Brueggeman, R., Muehlbauer, G., Wise, R., Wing, R., 2000. A bacterial artificial chromosome library for barley (*Hordeum vulgare* L.) and the identification of clones containing putative resistance genes. Theoret. Appl. Genet. 101, 1093–1099.

Ziegler, J., Wasternack, C., Hamberg, M., 1999. On the specificity of allene oxide cyclase. Lipids 34, 1005–1015.

Ziegler, J., Hamberg, M., Miersch, O., Parthier, B., 1997. Purification and characterization of allene oxide cyclase from dry corn seeds. Plant Physiol. 114, 565–573.

Ziegler, J., Stenzel, I., Hause, B., Maucher, H., Miersch, O., Hamberg, M., Grimm, M., Ganal, M., Wasternack, C., 2000. Molecular cloning of allene oxide cyclase: The enzyme establishing the stereochemistry of octadecanoids and jasmonates. J. Biol. Chem. 275, 19132–19138.