Wounding and chemicals induce expression of the *Arabidopsis thaliana* gene *Thi2.1*, encoding a fungal defense thionin, via the octadecanoid pathway

Holger Bohlmann[a,*], Alberto Vignutelli[a], Bernadette Hilpert[a], Otto Miersch[b], Claus Wasternack[b], Klaus Apel[a]

[a] *Swiss Federal Institute of Technology (ETH), Institute of Plant Sciences, ETH-Zentrum, Universitätstrasse 2, LFW D.58, CH-8092 Zürich, Switzerland*
[b] *Institut für Pflanzenbiochemie, Weinberg 3, D-06120 Halle (Saale), Germany*

Received 5 September 1998

Abstract In seedlings of *Arabidopsis thaliana* the thionin gene *Thi2.1* is inducible by methyl jasmonate, wounding, silver nitrate, coronatine, and sorbitol. We have used a biochemical and genetic approach to test the signal transduction of these different inducers. Both exogenously applied jasmonates and jasmonates produced endogenously upon stress induction, lead to GUS expression in a *Thi2.1* promoter-uidA transgenic line. No GUS expression was observed in a *coi1* mutant background which lacks jasmonate perception whereas methyl jasmonate and coronatine but not the other inducers were able to overcome the block in jasmonic acid production in a *fad3-2 fad7-2 fad8* mutant background. Our results show conclusively that all these inducers regulate *Thi2-1* gene expression via the octadecanoid pathway.

Key words: Octadecanoid pathway; Thionin; Jasmonate; Wounding

1. Introduction

Thionins are a group of usually basic, cysteine-rich peptides with toxic and antimicrobial properties (for a review, see [1–4]). Based on the toxicity against phytopathogenic bacteria, Fernandez de Caleya et al. [5] proposed a role for thionins in plant defense.

We have recently chosen *Arabidopsis thaliana* as an experimental system to further study the function of thionins. Expression of viscotoxin A3 from *Viscum album* in *A. thaliana* enhanced resistance against the soil-borne fungus *Plasmodiophora brassicae* [6]. Furthermore, we have identified two endogenous *A. thaliana* thionin genes that are regulated differently [7]. Induction of the *Thi2.1* gene correlates with resistance against *Fusarium oxysporum* f. sp. *matthiolae* [8]. Overexpression of THI2.1 in the susceptible ecotype Col-2 clearly enhanced the resistance of the transgenic lines [9].

The *Thi2.1* gene was found to be inducible by methyl jasmonate (MeJ), coronatine, silver nitrate, wounding, and pathogenic fungi, leading to local and systemic expression [7,10]. Salicylate and ethephon did not induce the *Thi2.1* gene, indicating that it is regulated via a signal transduction pathway different from that for PR proteins [7]. Jasmonic acid (JA) is though to mediate the wound signal transduction in plants which results in the expression of, among others, plant defense proteins such as proteinase inhibitors [11–13]. Furthermore, elicitation of a variety of plant cell cultures leads to a transient rise of endogenous 12-oxo-phytodienoic acid, an intermediate of jasmonate synthesis, followed by an endogenous rise of jasmonate and a subsequent synthesis of phytoalexins [14–16]. On the other hand, recent evidence indicates that wound-inducible genes in *A. thaliana* are regulated through JA-dependent and JA-independent pathways [17]. We have therefore tested if wounding and the different chemical inducers which we have identified for the *Thi2.1* gene are dependent on an endogenous rise of jasmonic acid. We demonstrate in this work that the *A. thaliana* thionin gene *Thi2.1* is solely regulated through the octadecanoid pathway after induction by wounding and the known chemical inducers. This conclusion is based on the use of inhibitors for the octadecanoid pathway, JA measurements, as well as of mutants which are insensitive to coronatine and MeJ [18] or defective in 18:3 fatty acid synthesis and thus unable to form JA [19].

2. Materials and methods

2.1. Chemicals

All inhibitors were obtained from Sigma. MeJ and sorbitol were bought from Fluka. Silver nitrate and linolenic acid were from Sigma.

2.2. Plant material

We used the *A. thaliana* ecotype Col-2. For seed production, plants were grown in soil in a greenhouse. The mutants *coi1* and *fad3-2 fad7-2 fad8* as well as the transgenic line *Thi2.1* GUS2.4 harboring a *Thi2.1* promoter-*uidA* construct were in the Columbia background. The *coi1* mutant was crossed with the *Thi2.1* promoter-*uidA* line. From this cross we selected lines which were homozygous for kanamycin resistance and segregating for *coi1*. Homozygous *fad3-2 fad7-2 fad8* mutants (detectable by their male sterile phenotype) were also crossed with the *Thi2.1* promoter-*uidA* line. 140 F2 plants each from 4 crosses were then grown to maturity. From these 4 different lines we obtained 8 male sterile plants. These plants were treated with 0.1% linolenic acid containing 0.05% Tween 20. Seeds of each plant were harvested separately and tested for kanamycin resistance. All lines were segregating for kanamycin resistance and 2 with a 3:1 segregation ($\chi^2 = 0.034$ and 0.004; $P > 0.95$) were selected for further studies.

2.3. Treatment of seedlings

Seeds were sterilized, sown on MS [20] plates with vitamins (nicotinic acid (0.5 mg/l), pyridoxine-HCl (0.5 mg/l), thiamine-HCl (0.1 mg/l)), glycerine (2 mg/l), 2% sucrose, and 0.8% agar, stored at 4°C for 2 days, and grown in a growth chamber (16 h light at 21°C, 8 h dark at 18°C) for 9 days. For tests of the *coi1* mutants 25 μM MeJ was included in the agar medium. For the coronatine experiment seedlings were grown in 24-well plates under otherwise the same conditions.

Seedlings were wounded by a V-shaped cut in one cotyledon as

*Corresponding author. Fax: (41) (1) 632-1081.
E-mail: holger.bohlmann@ipw.biol.ethz.ch

PII: S0014-5793(98)01251-4

described by Vignutelli et al. [10]. Concentrations of chemical inducers were as follows: MeJ was used as a 1 mM solution in 0.1% ethanol (v/v), silver nitrate at a concentration of 1 mM, and sorbitol as a 1 M solution. Coronatine (a gift from Dr. J. Turner) was used at a concentration of 100 nM. Other concentrations were as indicated. Seedlings were stained for GUS activity after 24 h.

2.4. GUS assays

GUS activity was determined with 4-methylumbelliferyl-β-D-glucuronide (MUG) according to Jefferson et al. [21] using 7-hydroxy-4-methylcoumarin (MU) as standard and is expressed as nM MU per minute per mg protein. Protein concentrations were measured according to Bradford [22].

In situ GUS staining was done as described by [21] with 5-bromo-4-chloro-3-indolylglucuronide (X-gluc) and plants were destained in 70% ethanol. GUS substrates were purchased from Biosynth (Staadt, Switzerland).

2.5. Jasmonate measurements

JA equivalents were determined using an ELISA as described by Lehmann et al. [23]. One gram fresh weight was used per sample. Data determined by this assay represent the sum of (−)-JA, and (−)-JA amino acid conjugates, calculated as JA equivalents.

3. Results

3.1. Induction of endogenous JA by silver nitrate and sorbitol

We have recently shown that the *A. thaliana Thi2.1* gene is inducible in seedlings by MeJ, coronatine, wounding, silver nitrate, and necrotrophic fungi [7,10]. Wounding of *A. thaliana* plants has been shown before to result in an increased endogenous level of jasmonic acid [24,25]. We have therefore tested if silver nitrate might have a similar effect. Fig. 1 shows that this inducer of the *Thi2.1* gene also leads to a strong increase in endogenous jasmonates already after 30 min with the highest level at 1 h after induction.

In addition to wounding, sorbitol treatment of barley leaves has also been previously shown to lead to a higher endogenous jasmonate level and subsequently to the activation of jasmonate inducible genes [26,23]. We therefore anticipated that sorbitol would also induce the *Thi2.1* gene. As shown in Fig. 2A, this is indeed the case. Sorbitol at 1 M induces the *Thi2.1* promoter-*uidA* transgene [10] to a level comparable to induction by 100 μM MeJ. We also found an elevated endogenous jasmonate level in the seedlings as demonstrated in Fig. 2B. Infiltration of 1 M sorbitol causes a significant rise in endogenous jasmonates starting after 2 h. The highest value was obtained after 6 h.

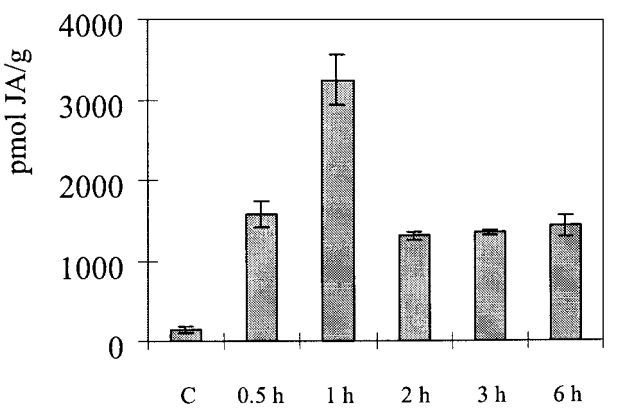

Fig. 1. Induction of endogenous JA by silver nitrate. Seedlings were grown on MS-agar plates containing 2% sucrose. After infiltration of a 1 mM silver nitrate solution, seedlings were harvested and ground in liquid nitrogen. Controls remained untreated. One gram of frozen powder was used for JA measurements. The experiment was repeated 2 times. Shown are the mean values of MeJ-equivalents in pmol/g fresh weight with standard deviation. C: Control.

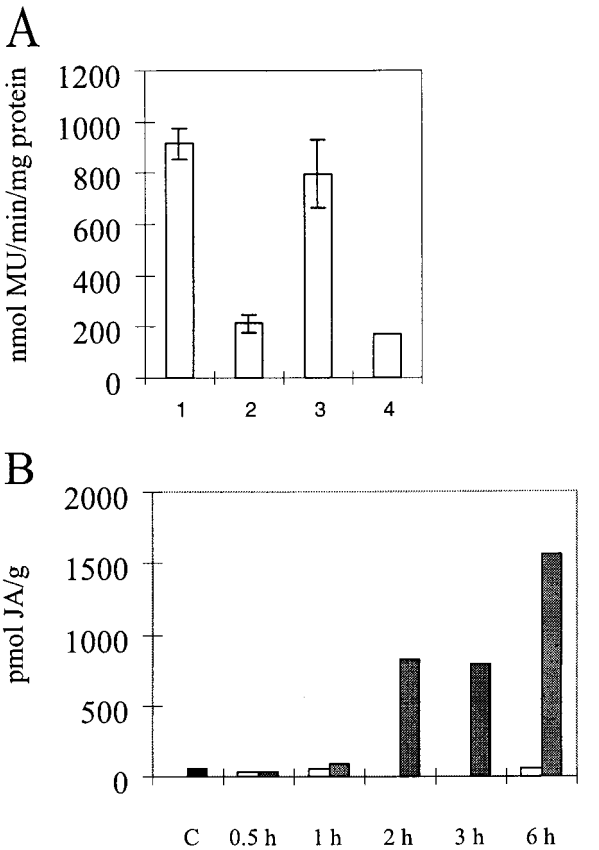

Fig. 2. Induction of the *Thi2.1* gene by sorbitol. A: Induction of GUS activity by sorbitol. Seedlings were grown on MS-agar plates for 9 days and infiltrated with the test solutions: 1, 1 M sorbitol; 2, 0.5 M sorbitol; 3, 100 μM MeJ; 4, control infiltrated with tap water. GUS activity was determined after 24 h and is given as nM MU/min/mg protein. Ten seedlings were used per measurement. The mean value of 3 experiments is given with standard deviation. B: Induction of JA by sorbitol. Seedlings were grown on MS-agar plates containing 2% sucrose. After infiltration of a 1 M sorbitol solution, seedlings were harvested and ground in liquid nitrogen (grey columns). Controls remained untreated. Mock infiltrations were done with sterile tap water (white columns). One gram of frozen powder was used for JA measurements. Shown are the values of MeJ-equivalents in pmol/g fresh weight. C: Control.

3.2. Inhibitors of JA biosynthesis lead to reduced Thi2.1 gene induction

Our results so far indicated an involvement of the octadecanoid pathway in the induction of the *Thi2.1* gene. We have therefore tested several inhibitors of JA biosynthesis if they affect the induction of the *Thi2.1* gene by wounding or silver nitrate treatment. We used a *Thi2.1* promoter-GUS line to measure the induction. GUS expression in this line has been shown before to closely mimic the expression of the endogenous *Thi2.1* gene [10]. We used the highest concentration of the inhibitors which could be included in the growth medium without inhibiting the growth of the seedlings (data not shown). At the concentrations used, most of these inhibitors significantly reduced the induction of the *Thi2.1* gene by silver nitrate and wounding as shown in Fig. 3A and B, respectively. Silver nitrate (at a concentration of 1 mM) induced the *Thi2.1*

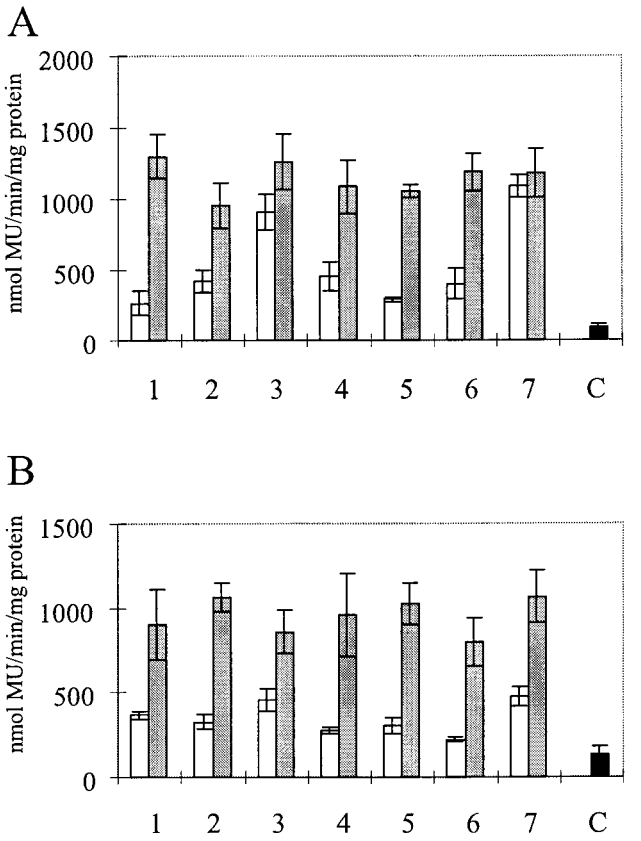

Fig. 3. Inhibitors of jasmonate biosynthesis reduce the induction of the *Thi2.1* gene by silver nitrate (A) and wounding (B). Transgenic seedlings harboring a *Thi2.1* promoter-*uidA* construct were grown on MS medium. Inhibitors were included in the agar at the following concentrations: aspirin, 10 μM; salicylate, 10 μM; piroxicam, 10 μM; *n*-propyl-gallate, 100 μM; indomethacin, 10 μM; ibuprofen, 10 μM. Nine days old seedlings were induced by infiltration of 1 mM silver nitrate (A) or by cutting a cotyledon (B). Controls were treated with water (black columns). White columns indicate GUS expression after induction by silver nitrate or wounding. Part of the seedlings were additionally infiltrated with 100 μM MeJ (grey columns). Shown are mean values of six experiments with standard deviation for GUS expression (nM MU/min/mg protein). 1: Aspirin; 2: salicylate; 3: piroxicam; 4: *n*-propyl-gallate; 5: indomethacin; 6: ibuprofen; 7: no inhibitor; C: water treated controls.

gene to a higher level than wounding (see also [10]). Therefore, the inhibition is also more pronounced in the case of silver nitrate.

In the case of silver nitrate treatment, addition of exogenous MeJ at 100 μM was able to overcome the effect of all inhibitors and gave an expression level comparable to control seedlings grown without inhibitors. In the case of wounded seedlings, MeJ more than compensated for the reduction by the different inhibitors due to the lower induction level by wounding [10]. These results indicated that inhibition is exerted in the pathway before the production of JA.

3.3. JA mutants

Further evidence that the induction of the *Thi2.1* gene is mediated by the octadecanoid pathway was obtained by using two mutants of the octadecanoid pathway. The *coi1* mutation is recessive and leads to male sterility and insensitivity to coronatine and MeJ [18]. The *fad3-2 fad7-2 fad8* mutant [19] has no detectable linolenic acid (18:3) and is unable to produce jasmonate. It is therefore also male sterile and compromised in the expression of wound inducible genes [24]. Both mutants were crossed with a *Thi2.1* promoter-*uidA* line as described in Section 2. Seedlings were tested for GUS expression after treatment with different inducers of the *Thi2.1* gene.

Due to the male sterility of the *coi1* mutant it was not possible to obtain homozygous populations. Furthermore, the inducibility of the *Thi2.1* gene is most pronounced in young seedlings [10] and since the effect of preselection with MeJ is not known, we had to work with populations segregating for *coi1*. As shown in Table 1, two lines were used that were homozygous for kanamycin resistance (which is linked to the *Thi2.1* promoter-*uidA* cassette) and segregated 3:1 for sensitivity and insensitivity to MeJ when grown on agar medium containing MeJ. Hence, 25% of the seedlings should be homozygous for *coi1* and thus should be blocked in the expression of genes regulated by the octadecanoid pathway. Homozygous *coi1* mutants, which had a normal phenotype on MeJ, never showed any GUS staining. In contrast, seedlings with the typical growth inhibition known from wild-type plants always showed a strong GUS staining (data not shown). A quantitative evaluation of these experiments is shown in Table 2 for MeJ and all other inducers. Two different populations homozygous for the *Thi2.1* promoter-*uidA* transgene but segregating for *coi1* were used. GUS expression in these seedlings segregated 3:1 after wounding or treatment with chemical inducers. Control experiments were performed with the *Thi2.1* promoter-*uidA* transgene in the Columbia background (*Thi2.1* GUS). GUS expression in the *Thi2.1* GUS line was clearly detectable in all seedlings following the different inducer treatments but not after infiltration of tap water. These data show that the *Thi2.1* gene is not inducible in homozygous *coi1* seedlings. Thus, induction by JA, coronatine, wounding, silver nitrate, and sorbitol is most likely regulated exclusively via the octadecanoid pathway.

Seedlings of the *fad3-2 fad7-2 fad8* mutant which also contained the *Thi2.1* promoter-*uidA* transgene were similarly tested for GUS expression after induction. While being homozygous for *fad3-2 fad7-2 fad8* (male sterility in these mutants can be overcome by feeding the flower buds with linolenic

Table 1
Segregation of kanamycin resistance and *coi1* phenotype in two *coi1-Thi2.1* GUS lines

Line	Kan^R:Kan^S	χ^2	MeJ^S:MeJ^I	Segregation of MeJ^S	χ^2
1	218:1	0.01	127:57	2.23:1	3.51
12	193:1	0.01	64:22	2.91:1	0.02

Seedlings were grown on MS-agar plates containing 50 μg/ml kanamycin or 25 μmol MeJ. After 2 weeks, seedlings were evaluated for kanamycin resistance and growth inhibition by MeJ. Kan^R: kanamycin resistant; Kan^S: kanamycin susceptible; MeJ^S: growth inhibition by MeJ; MeJ^I: no growth inhibition by MeJ. χ^2 test showed that the lines are homozygous for kanamycin resistance and segregate 3:1 for the *coi1* phenotype ($P > 0.95$).

Table 2
Expression of GUS segregates 3:1 in *coi1-Thi2.1* GUS lines

Line	Treatment	Blue	White	Segregation	χ^2
*coi1-Thi2.1*GUS.01	Tap water	4	238	–	–
*coi1-Thi2.1*GUS.12	Tap water	2	193	–	–
*Thi2.1*GUS	Tap water	0	214	–	–
*coi1-Thi2.1*GUS.01	Wounding	103	37	2.78:1	0.15
*coi1-Thi2.1*GUS.12	Wounding	74	25	2.96:1	0.01
*Thi2.1*GUS	Wounding	63	0	–	–
*coi1-Thi2.1*GUS.01	MeJ	112	32	3.5:1	0.59
*coi1-Thi2.1*GUS.12	MeJ	181	62	2.92:1	0.03
*Thi2.1*GUS	MeJ	173	0	–	–
*coi1-Thi2.1*GUS.01	Silver nitrate	192	74	2.59:1	1.13
*coi1-Thi2.1*GUS.12	Silver nitrate	90	26	3.46:1	0.41
*Thi2.1*GUS	Silver nitrate	214	0	–	–
*coi1-Thi2.1*GUS.01	Coronatine	92	33	2.79:1	0.13
*coi1-Thi2.1*GUS.12	Coronatine	148	50	2.96:1	0.01
*Thi2.1*GUS	Coronatine	106	0	–	–
*coi1-Thi2.1*GUS.01	Sorbitol	141	53	2.66:1	0.56
*coi1-Thi2.1*GUS.12	Sorbitol	133	47	2.83:1	0.12
*Thi2.1*GUS	Sorbitol	121	1	–	–

Seedlings were grown on MS-agar plates and induced as described in Section 2. After X-gluc staining, white seedlings and those with blue color were counted and a segregation ratio determined. χ^2 test showed that the lines segregate 3:1 for GUS induction with the different inducers ($P > 0.95$).

acid), they were segregating for kanamycin resistance. Seedlings of two different lines were therefore plated on kanamycin containing media and only Kan^R seedlings were analyzed. As a control, we used the corresponding F2 populations from which the homozygous *fad3-2 fad7-2 fad8* mutants had been obtained. These F2 populations are segregating for the 3 *fad* mutations and statistically only 1 in 64 should be homozygous for all three mutations, which is neglectable. Treatments which have been shown to lead to higher jasmonate levels in plants such as wounding, silver nitrate and sorbitol infiltration do not induce the *Thi2.1* promoter-*uidA* transgene in the *fad3-2 fad7-2 fad8* background as documented in Table 3. However, MeJ and coronatine were strong inducers.

4. Discussion

JA has important and diverse roles in plant growth and development and is produced in plants from linolenic acid via octadecanoids [13,27,28]. JA levels increase after wounding [24,25,29–31], elicitor treatment [14,16], and sorbitol stress [23]. For 12-oxo-phytodienoic acid, an intermediate of JA synthesis, a transient accumulation preceding that of JA has been described following elicitation of cell cultures [16] and wounding [25]. Several JA-insensitive mutants have been isolated which overcome the JA inhibition of root growth in *Arabidopsis* [18,32,33]. However, not much is known at present about how JA is perceived and transduced to induce specific genes. One component that has been identified in the JA signal transduction pathway is reversible protein phosphorylation [17]. Other authors have indicated an involvement of MAP kinases upstream of jasmonate [34,35].

4.1. The Thi2.1 gene is induced through the octadecanoid pathway

The *Arabidopsis* thionin gene *Thi2.1* is constitutively expressed in ovaries and siliques [7,10]. In seedlings, expression of the gene is not detectable by RNA blot analysis if plants are grown under sterile conditions, but is induced by necrotrophic fungi, MeJ, silver nitrate, coronatine, sorbitol, and wounding ([7,10], this work). Using a *Thi2.1* promoter-GUS line we have demonstrated in this work that the induction by wounding or chemicals is solely regulated through the octadecanoid pathway. GUS expression in this line has been shown before to faithfully mimic the expression of the endogenous *Thi2.1* gene [10].

A time-dependent rise of endogenous jasmonates has been directly measured after treatment with silver nitrate and sorbitol. The latter has been shown before to increase the endog-

Table 3
Expression of GUS *fad3-2 fad7-2 fad8*-GUS lines

	TRI-GUS1.A[a] (homozygous)		TRI-GUS19.B[a] (homozygous)		TRI-GUS1[b] (segregating)		TRI-GUS19[b] (segregating)	
	Blue	White	Blue	White	Blue	White	Blue	White
Untreated	0	9	0	11	0	21	0	16
MeJ	13	0	15	0	18	0	19	0
Coronatine	7	0	9	0	7	0	11	0
Wounding	0	12[c]	0	15[c]	14	0	10	0
Silver nitrate	0	19	0	22	19	0	15	0
Sorbitol	0	21	0	16	13	0	9	0

Seedlings were grown on MS-agar plates and induced as described in Section 2. After X-gluc staining, white seedlings and those with blue color were counted.
[a]Homozygous for *fad3-2 fad7-2 fad8*.
[b]Controls segregating for the 3 fad mutations.
[c]Some of the seedlings showed a very weak blue staining in the apical region.

enous levels of JA and its amino acid conjugates and subsequently to induce JA-responsive genes in barley leaves [23,26,36]. Wounding has also been shown by others to increase endogenous jasmonate levels in *Arabidopsis* [24,25], soybean [29], or tomato [30,31,37]. This wound-induced elevation of jasmonates in *Arabidopsis* corresponds with the wound-induced expression of genes coding for jasmonate-biosynthetic enzymes such as lipoxygenase [38] or allene oxide synthase [39].

The view that the induction of the *Thi2.1* gene is mediated by JA produced in response to wounding and silver nitrate is further supported by inhibitor experiments. Aspirin, salicylate, propylgallate, indomethacin, and ibuprofen all resulted in a reduced GUS expression level after wounding or silver nitrate infiltration. These drugs are suggested to inhibit lipoxygenase or hydroperoxide dehydrase, respectively, which are involved in the biosynthesis of JA [37,40–42]. However, none of these inhibitors blocked the *Thi2.1* induction completely. The reason for this is not clear, but may be related to the application of the inhibitors in the growth medium. We used only those concentrations which had no visible effect on the growth of the seedlings. Considering that the uptake through the roots might not have been optimal, the actual concentrations in the seedlings might have been too low. Nevertheless, the results are in line with the other approaches which we have taken. Piroxicam was not effective as an inhibitor in our system. Whether this may be due to the application in the growth medium is not known. Aspirin and salicylate have both been shown to inhibit wound-induced gene expression in tomato by blocking the biosynthesis of jasmonic acid [37], but inhibition downstream of JA has also been observed [42].

All known inducers were not effective in the *coi1* mutant which is blocked in the perception of coronatine and jasmonates. The *COI1* gene has recently been cloned and appears to be an F-box protein which may target repressor proteins for removal by ubiquitination [43]. These authors have also shown that induction of the *Thi2.1* gene by wounding and MeJ is dependent on *COI1* [43]. It is assumed that *COI1* acts on a protein phosphatase 2A [17].

Our results using the *fad3-2 fad7-2 fad8* mutant [19] showed that the inducers can be divided into two groups. The first group comprises MeJ and coronatine which is discussed to be a molecular mimic of 12-oxo-phytodienoic acid [44] or JA amino acid conjugates [45,46]. MeJ and coronatine were still effective in the *fad3-2 fad7-2 fad8* mutant, and this result is in line with the block in this mutant being in JA production and not perception. The second group includes inducers and treatments that lead to an endogenous rise of jasmonates in the seedlings. Wounding as well as sorbitol and silver nitrate infiltration did not induce the *Thi2.1* promoter-GUS construct in the *fad3-2 fad7-2 fad8* mutant background due to the block in JA biosynthesis. Our data support the assumption that signalling via exogenously applied JA and endogenously produced JA converge to induce the *Thi2.1* gene. In contrast, other JA-responsive genes such as those coding for a lipoxygenase of barley [47] or a caffeic acid *O*-methyltransferase from barley [48] are inducible by exogenous JA only.

Taken together, we have demonstrated by JA measurements, inhibitor experiments, and the use of two different JA mutants, that induction of the *Thi2.1* gene by wounding and chemical inducers is mediated through the octadecanoid pathway. Furthermore, our results indicated that induction of the *Thi2.1* gene by necrotrophic fungi such as *Fusarium oxysporum* f. sp. *matthiolae* [7,10] may also be mediated through the octadecanoid pathway. Results concerning this subject will be dealt with in a separate publication.

4.2. Constitutive expression in ovaries and siliques

We have recently shown that siliques contain a high constitutive level of jasmonates [10]. This might explain the strong constitutive expression of the *Thi2.1* gene in ovaries and siliques [7,10]. Furthermore, promoter analysis has shown that a promoter region of less than 100 bp is solely responsible for inducible and constitutive expression of the *Thi2.1* promoter [10]. Mutant analysis of the constitutive expression in seedlings is hampered by the fact that deficiency in jasmonate biosynthesis as in the *fad3-2 fad7-2 fad8* mutant [19] and deficiency in the JA response in the *coi1* mutant [18] are linked with male sterility. This makes it impossible to obtain homozygous *coi1* siliques. Siliques from homozygous *fad3-2 fad7-2 fad8* mutants can only be obtained by feeding jasmonate or a precursor. Siliques obtained this way from plants containing the *Thi2.1* promoter-GUS construct always stained blue (data not shown). Ovaries from *coi1* and *fad3-2 fad7-2 fad8* mutants containing the *Thi2.1* promoter-GUS construct showed a slight GUS staining, although much weaker than in the wild-type background (data not shown). This may be due to a slight leakiness of the mutants, although the *fad3-2 fad7-2 fad8* mutant has been reported to contain no detectable linolenic acid [19]. Thus, the strong constitutive expression of the *Thi2.1* gene in ovaries and siliques is probably also due to elevated JA levels in these tissues.

Acknowledgements: This work was supported by the Swiss National Science Foundation. We are indebted to Drs. J. Turner and J. Browse for the *coi1* and *fad3-2 fad7-2 fad8* mutants, respectively. We thank Mrs. C. Gebhardt (Halle) for JA measurements.

References

[1] Garcia-Olmedo, F., Rodriguez-Palenzuela, P., Hernandez-Lucas, C., Ponz, F., Marana, C., Carmona, M.J., Lopez-Fando, J., Fernandez, J.A. and Carbonero, P. (1989) Oxf. Surv. Plant Mol. Cell Biol. 6, 31–60.
[2] Bohlmann, H. and Apel, K. (1991) Annu. Rev. Plant Phys. Plant Mol. Biol. 42, 227–240.
[3] Bohlmann, H. (1994) Crit. Rev. Plant Sci. 13, 1–16.
[4] Florack, D.E.A. and Stiekema, W.J. (1994) Plant Mol. Biol. 26, 25–37.
[5] Fernandez de Caleya, R., Gonzalez-Pascual, B., Garcia-Olmedo, F. and Carbonero, P. (1972) Appl. Microbiol. 23, 998–1000.
[6] Holtorf, S., Ludwig-Müller, J., Apel, K. and Bohlmann, H. (1998) Plant Mol. Biol. 36, 673–680.
[7] Epple, P., Apel, K. and Bohlmann, H. (1995) Plant Physiol. 109, 813–820.
[8] Epple, P., Vignutelli, A., Apel, K. and Bohlmann, H. (1998) Mol. Plant-Microbe Interact. 11, 523–529.
[9] Epple, P., Apel, K. and Bohlmann, H. (1997) Plant Cell 9, 509–520.
[10] Vignutelli, A., Wasternack, C., Apel, K. and Bohlmann, H. (1998) Plant J. 14, 285–295.
[11] Bergey, D.R., Howe, G.A. and Ryan, C.A. (1996) Proc. Natl. Acad. Sci. USA 93, 12053–12058.
[12] Koiwa, H., Bressan, R.A. and Hasegawa, P.M. (1997) Trends Plant Sci. 2, 379–384.
[13] Wasternack, C. and Parthier, B. (1997) Trends Plant Sci. 2, 302–307.
[14] Gundlach, H., Müller, M.J., Kutchan, T.M. and Zenk, M.H. (1992) Proc. Natl. Acad. Sci. USA 89, 2389–2393.

[15] Blechert, S., Brodschelm, W., Hölder, S., Kammerer, L., Kutchan, T.M., Mueller, M.J., Xia, Z.-Q. and Zenk, M.H. (1995) Proc. Natl. Acad. Sci. USA 92, 4099–4105.
[16] Parchmann, S., Gundlach, H. and Mueller, M.J. (1997) Plant Physiol. 115, 1057–1064.
[17] Rojo, E., Titarenko, E., León, J., Berger, S., Vancanneyt, G. and Sánchez-Serrano, J. (1998) Plant J. 13, 153–165.
[18] Feys, B.J.F., Benedetti, C.E., Penfold, C.N. and Turner, J.G. (1994) Plant Cell 6, 751–759.
[19] McConn, M. and Browse, J. (1996) Plant Cell 8, 403–416.
[20] Murashige, T. and Skoog, F. (1962) Physiol. Plant 15, 473–497.
[21] Jefferson, R.A., Kavanagh, T.A. and Bevan, M. (1987) EMBO J. 6, 3901–3907.
[22] Bradford, M.M. (1979) Anal. Biochem. 72, 248–254.
[23] Lehmann, J., Atzorn, R., Brückner, C., Reinbothe, S., Leopold, J., Wasternack, C. and Parthier, B. (1995) Planta 197, 156–162.
[24] McConn, M., Creelman, R.A., Bell, E., Mullet, J.E. and Browse, J. (1997) Proc. Natl. Acad. Sci. USA 94, 5473–5477.
[25] Weber, H., Vick, B.A. and Farmer, E.E. (1997) Proc. Natl. Acad. Sci. USA 94, 10473–10478.
[26] Kramell, R., Atzorn, R., Schneider, G., Miersch, O., Brückner, C., Schmidt, J., Sembdner, G. and Parthier, B. (1995) J. Plant Growth Regul. 14, 29–36.
[27] Creelman, R.A. and Mullet, J.E. (1995) Proc. Natl. Acad. Sci. USA 92, 4114–4119.
[28] Creelman, R.A. and Mullet, J.E. (1997) Annu. Rev. Plant Physiol. Plant Mol. Biol. 48, 355–381.
[29] Creelman, R.A., Tierney, M.L. and Mullet, J.E. (1992) Proc. Natl. Acad. Sci. USA 89, 4938–4941.
[30] Conconi, A., Miquel, M., Browse, J.A. and Ryan, C.A. (1996) Plant Physiol. 111, 797–803.
[31] O'Donnell, P.J., Calvert, C., Atzorn, R., Wasternack, C., Leyser, H.M.O. and Bowles, D.J. (1996) Science 274, 1914–1917.
[32] Staswick, P.E., Su, W. and Howell, S.H. (1992) Proc. Natl. Acad. Sci. USA 89, 6837–6840.
[33] Berger, S., Bell, E. and Mullet, J.E. (1996) Plant Physiol. 111, 525–531.
[34] Seo, S., Okamoto, M., Seto, H., Ishizuka, K., Sano, H. and Ohashi, Y. (1995) Science 270, 1988–1992.
[35] Usami, S., Banno, H., Ito, Y., Nishihama, R. and Machida, Y. (1995) Proc. Natl. Acad. Sci. USA 92, 8660–8664.
[36] Kramell, R., Miersch, O., Hause, B., Ortel, B., Parthier, B. and Wasternack, C. (1997) FEBS Lett. 414, 197–202.
[37] Peña-Cortes, H., Albrecht, T., Prat, S., Weiler, E.W. and Willmitzer, L. (1993) Planta 191, 123–128.
[38] Bell, E., Creelman, R.A. and Mullet, J.E. (1995) Proc. Natl. Acad. Sci. USA 92, 8675–8679.
[39] Laudert, D., Pfannschmidt, U., Lottspeich, F., Hollaender-Czytko, H. and Weiler, E.W. (1996) Plant Mol. Biol. 31, 323–335.
[40] Doherty, H.M., Selvendran, R.R. and Bowles, D.J. (1988) Physiol. Mol. Plant Pathol. 33, 377–384.
[41] Staswick, P.E., Huang, J.-F. and Rhee, Y. (1991) Plant Physiol. 96, 130–136.
[42] Doares, S.H., Narváez-Vásquez, J., Conconi, A. and Ryan, C. (1995) Plant Physiol. 108, 1741–1746.
[43] Xie, D.-X., Feys, B.F., James, S., Nieto-Rostro, M. and Turner, J.G. (1998) Science 280, 1091–1094.
[44] Weiler, E.W., Kutchan, T.M., Gorba, T., Brodschelm, W., Niesel, U. and Bublitz, F. (1994) FEBS Lett. 345, 9–13.
[45] Krumm, T., Bandemer, K. and Boland, W. (1995) FEBS Lett. 377, 523–529.
[46] Wasternack, C., Ortel, B., Miersch, O., Kramell, R., Beale, M., Greulich, F., Feussner, I., Hause, B., Krumm, T., Boland, W. and Parthier, B. (1998) J. Plant Physiol. 152, 345–352.
[47] Vörös, K., Feussner, I., Kühn, H., Lee, J., Graner, A., Löbler, M., Parthier, B. and Wasternack, C. (1998) Eur. J. Biochem. 251, 36–44.
[48] Lee, J., Parthier, B. and Löbler, M. (1996) Planta 199, 625–632.