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RESEARCH PAPER

TGA transcription factors and jasmonate-independent COI1 signalling regulate specific plant responses to reactive oxylipins

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Abstract

Jasmonates and phytoprostanes are oxylipins that regulate stress responses and diverse physiological and developmental processes. 12-Oxo-phytodienoic acid (OPDA) and phytoprostanes are structurally related electrophilic cyclopentenones, which activate similar gene expression profiles that are for the most part different from the action of the cyclopentanone jasmonic acid (JA) and its biologically active amino acid conjugates. Whereas JA–isoleucine signals through binding to COI1, the bZIP transcription factors TGA2, TGA5, and TGA6 are involved in regulation of gene expression in response to phytoprostanes. Here root growth inhibition and target gene expression were compared after treatment with JA, OPDA, or phytoprostanes in mutants of the COI1/MYC2 pathway and in different TGA factor mutants. Inhibition of root growth by phytoprostanes was dependent on COI1 but independent of jasmonate biosynthesis. In contrast, phytoprostane-responsive gene expression was strongly dependent on TGA2, TGA5, and TGA6, but not dependent on COI1, MYC2, TGA1, and TGA4. Different mutant and overexpressing lines were used to determine individual contributions of TGA factors to cyclopentenone-responsive gene expression. Whereas OPDA-induced expression of the cytochrome P450 gene *CYP81D11* was primarily regulated by TGA2 and TGA5, the glutathione *S*-transferase gene *GST25* and the OPDA reductase gene *OPR1* were regulated by TGA5 and TGA6, but less so by TGA2. These results support the model that phytoprostanes and OPDA regulate differently (i) growth responses, which are COI1 dependent but jasmonate independent; and (ii) lipid stress responses, which are strongly dependent on TGA2, TGA5, and TGA6. Identification of molecular components in cyclopentenone signalling provides an insight into novel oxylipin signal transduction pathways.

Key words: *Arabidopsis thaliana*, biotic and abiotic stress, class II TGA factors, detoxification, lipid signaling, reactive electrophile oxylipins.

Introduction

Oxygenation of polyunsaturated fatty acids leads to the production of oxylipins, such as jasmonates and phytoprostanes, via enzymatic or non-enzymatic pathways (Mueller, 2004; Wasternack, 2007). Exogenous application of jasmonic acid (JA) inhibits mitosis, root growth, and seed germination (Swiatek *et al*., 2002). Endogenous jasmonate biosynthesis is required for development of fertile flowers (Sanders *et al*., 2000). Jasmonates also control abiotic and biotic stress responses with a concomitant induction of a variety of genes related to JA biosynthesis and defence (Devoto *et al*., 2005). Biological activities have also been reported for 12-oxo-phytodienoic acid (OPDA), which is a precursor

Abbreviations: AOS, allene oxide synthase; JA, jasmonic acid; JAZ, JASMONATE ZIM-domain; OPDA, 12-oxo-phytodienoic acid; PGA_1, prostaglandin A_1; SA, salicylic acid.

of JA biosynthesis. OPDA inhibits root growth and mitosis similarly to JA but induces a different set of genes (Taki *et al.*, 2005; Mueller *et al.*, 2008). Endogenous OPDA was recently shown to impede seed germination independent of JA biosynthesis and signalling (Dave *et al.*, 2011; Dave and Graham, 2012). Mutants with defects in oxylipin biosynthesis, signalling, and transport were used to establish the biological functions of both compounds (McConn and Browse, 1996; McConn *et al.*, 1997; Stintzi and Browse, 2000; Malek *et al.*, 2002; Park *et al.*, 2002; Mene-Saffrane *et al.*, 2009; Dave *et al.*, 2011; Stotz *et al.*, 2011). Such studies demonstrated that jasmonates protect plants against chewing insects (Howe *et al.*, 1996; McConn *et al.*, 1997; Pieterse *et al.*, 2012) and modulate host–pathogen interactions (Ton *et al.*, 2002; Laurie-Berry *et al.*, 2006; Pieterse *et al.*, 2012). OPDA was shown to protect specifically against necrotrophic pathogens and not by virtue of its being a JA precursor (Raacke *et al.*, 2006; Stotz *et al.*, 2011).

Phytoprostanes are non-enzymatically formed compounds with structural similarity to OPDA (Mueller, 2004). Similarly to JA and OPDA, these compounds inhibit root growth and mitosis and induce the production of secondary metabolites (Mueller *et al.*, 2008). The set of genes which is induced by phytoprostanes shows a strong overlap with the OPDA-responsive genes and only a small overlap with JA-induced genes. This can be explained by the presence of an α,β-unsaturated carbonyl group in OPDA and phytoprostanes, which are electrophilic cyclopentenones. In contrast, JA is a non-electrophilic and chemically unreactive cyclopentanone. The α,β-unsaturated carbonyl group is the reason for the higher chemical reactivity, which was suggested to be crucial for the biological activity (Farmer and Davoine, 2007).

Recently, substantial progress has been made towards understanding the signal transduction pathway mediating the response to jasmonates. JA–isoleucine (JA-Ile), the biologically active form of JA, is bound to the F-box protein COI1 in the presence of JASMONATE ZIM-domain (JAZ) protein family members (Chini *et al.*, 2007; Thines *et al.*, 2007; Sheard *et al.*, 2010). JAZ proteins act as negative regulators of jasmonate-responsive gene expression. Binding of JA-Ile leads to the degradation of JAZ proteins, resulting in the release of transcription factors such as MYC2, which promote the expression of jasmonate-responsive genes (Chini *et al.*, 2007). *MYC2* was identified via positional cloning of a jasmonate-insensitive *jin1* mutant allele (Berger *et al.*, 1996); *JIN1* encodes the basic helix–loop–helix transcription factor MYC2 (Lorenzo *et al.*, 2004).

In contrast to the jasmonate signal transduction pathway, only little is known about the mechanism that mediates the effects of OPDA and phytoprostanes. Putative binding sites for TGA transcription factors are over-represented in promoters of phytoprostane-responsive genes, and specifically the TGA2, TGA5, and TGA6 factors were shown to regulate gene expression in response to cyclopentenone oxylipins (Mueller *et al.*, 2008). Induction of 30% and 60% of the genes in response to OPDA and the phytoprostane PPA_1, respectively, did not occur in the *tga2 tga5 tga6* mutant, which is defective in expression of all three *TGA* factor genes. However, the participation of other TGA factors in responses to these cyclopentenones has not been tested.

The primary aim of this study was to uncover signalling pathways that mediate the effects of reactive oxylipins on plant growth and stress responses, the jasmonate receptor COI1 and TGA transcription factors being of particular interest. With respect to stress responses, specific contributions of individual TGA factors to OPDA-dependent gene expression were determined using the cytochrome P450 gene *CYP81D11*, the regulation of which was further characterized recently (Köster *et al.*, 2012), the glutathione *S*-transferase gene *GST25*, and the OPDA reductase gene *OPR1*.

Materials and methods

Plant material and growth conditions

The *jin1* and *coi1-16* mutants together with their *Arabidopsis thaliana* (L.) Heynh. background Col-*gl* were those originally reported (Berger *et al.*, 1996; Ellis and Turner, 2002; Nickstadt *et al.*, 2004). The *dde2-2* mutant in the background of ecotype Col-0 was previously published (Malek *et al.*, 2002). The *tga6*, *tga2 tga5*, and *tga2 tga5 tga6* mutants as well as the *tga1 tga4* double mutant were those originally described (Zhang *et al.*, 2003; Kesarwani *et al.*, 2007). All transgenic lines overexpressing *TGA2*, *TGA5*, or *TGA6* were received from Professor Christiane Gatz. In addition to the previously published lines *TGA2.1*, *TGA2.2*, *TGA5.1*, *TGA5.2*, and *TGA6.2* (Zander *et al.*, 2010), novel *TGA5* and *TGA6* lines were tested. All *tga* mutant and *TGA*-overexpressing lines were generated in the background of ecotype Col-0.

Seedlings were grown in liquid MS (Murashige and Skoog) medium containing 1% or 2% sucrose or on MS agar plates as previously described (Mueller *et al.*, 2008). Seedlings were grown with a 9 h light/15 h dark cycle at 22 °C under fluorescent light (150 µmol m^{-2} s^{-1}).

Chemical treatments

Seedlings grown in liquid MS medium or on MS agar plates were treated with OPDA synthesized by enzymatic conversion of linolenic acid using linseed acetone powder (Parchmann *et al.*, 1997), JA (Sigma-Aldrich, St Louis, MO, USA), the phytoprostane PPA_1 (Thoma *et al.*, 2003), or the prostaglandin PGA_1 (Cayman Chemical, Ann Arbor, MI, USA).

Quantitative real-time PCR analysis

Total RNA was extracted from liquid-grown seedlings using the E.Z.N.A. plant RNA kit (Omega Bio-Tek, Norcross, GA, USA). Potential DNA contamination was removed using on-column digestion with DNase I. Following quantification using an ND-1000 UV-Vis Spectrophotometer (NanoDrop, Wilmington, DE, USA), 1 µg of total RNA was used for cDNA synthesis using M-MLV RNase H minus reverse transcriptase (Promega, Madison, WI, USA). Real-time PCR was performed using a QPCR SYBR Green Mix (Thermo Scientific, Lafayette, CO, USA). Primers are listed in Supplementary Table S1 available at *JXB* online, except for *OPR1* and *Act2/8*, which have already been published (Mueller *et al.*, 2008; Ellinger *et al.*, 2010). Reactions were performed on a Mastercycler Realplex (Eppendorf, Wesseling-Berzdorf, Germany) or on a CFX96 Real-Time PCR Detection System (BioRad, Hercules, CA, USA) with 40 cycles of denaturation for 15 s at 95 °C, annealing for 20 s at 55 °C, and extension for 20 s at 72 °C. This program was followed by a melting curve analysis. Purified real-time PCR products were used for calibration using the relative standard curve method

(Appplied Biosystems, Carlsbad, CA, USA). Three biological replicates were used for each data point.

Statistical analysis

Analysis of variance (ANOVA) was used for statistical analysis of root growth measurements. Levene's test was used to determine homogeneity of variances. Data were transformed to achieve homogeneous variances. Alternatively, data were analysed using non-parametric statistics. Two-tailed tests were used with $\alpha < 0.05$. The Relative Expression Software Tool V2.0.13 (Qiagen, Hilden, Germany) was used to determine the significance of pairwise comparisons of quantitative PCR data.

Results

Inhibition of root growth by phytoprostanes is dependent on COI1 but independent of jasmonate biosynthesis

An effect shared by jasmonates and phytoprostanes is the inhibition of root growth, which was previously measured in wild-type *A. thaliana* seedlings after treatment with OPDA or PPA_1 (Mueller *et al*., 2008). COI1 is known to mediate inhibition of root growth in response to exogenous JA or JA methyl ester. To test whether inhibition of root growth in response to phytoprostanes is also COI1 dependent, the response of the *coi1* mutant was analysed. The root length of *coi1* seedlings on medium containing 25 µM JA, OPDA, or PPA_1 was similar to that of the control grown on MS medium without the addition of oxylipins (Fig. 1A). This demonstrates that inhibition of root growth by OPDA or phytoprostanes is dependent on COI1. In addition, this result shows that growth inhibition is not based on a toxic effect of cyclopentenones but on signalling processes.

It is not clear whether OPDA exerts the observed effect directly or indirectly via JA biosynthesis because the *coi1* mutant can convert OPDA to JA. So far, COI1 has only been shown to bind amino acid conjugates of JA and coronatine (Thines *et al*., 2007; Katsir *et al*., 2008). This raises the question of whether JA-Ile mediates the effect of PPA_1. To investigate the possibility that an accumulation of JA-Ile upon PPA_1 treatment is responsible for the inhibition of root growth, the *dde2* mutant was tested. This mutant contains a knockout allele of the allene oxide synthase (AOS) gene (Malek *et al*., 2002). As a result, the *dde2* mutant no longer produces OPDA, JA, and JA-Ile (Köster *et al*., 2012). Inhibition of root growth in the *dde2* mutant in response to phytoprostane treatment was similar to the root growth inhibition observed in the wild type (Table 1). This clearly shows that the inhibitory effect of phytoprostanes on root growth is not mediated through OPDA or JA-Ile. These data also demonstrate that COI1 plays an important role in mediating root growth-inhibitory effects of oxylipins other than jasmonates.

As mentioned above, induction of gene expression in response to cyclopentenones is impaired in the *tga2 tga5 tga6* mutant. It was therefore investigated whether this mutant is also insensitive to oxylipin-triggered inhibition of root growth. On control medium without oxylipins, roots of the *tga2 tga5 tga6* mutant were considerably shorter (54%) than wild-type roots ($F_{1,132}$=230.6, $P < 0.001$). Oxylipins strongly inhibited root growth. Root growth of the *tga2 tga5 tga6* mutant was more sensitive to the presence of PPA_1 ($F_{1,198}$=42.4, $P < 0.001$) and JA ($F_{1,208}$=5.3, $P = 0.023$) than wild-type roots (Fig. 1B). The difference in genotype-dependent inhibition of root growth by OPDA was not significantly different. Root lengths of the triple mutant were reduced to 15, 21, and 26% relative to the lengths on control medium in the presence of PPA_1, OPDA, and JA, respectively; corresponding relative root lengths in the wild type were 56, 27, and 35%. These data illustrate that the transcription factors TGA2, TGA5, and TGA6 are not required for root growth inhibition in response to oxylipins. Instead, the *tga2 tga5 tga6* mutant was particularly hypersensitive to PPA_1.

Root growth was also analysed in *tga1 tga4*, a double mutant defective in expression of TGA1 and TGA4, which represents a different class of TGA factors. In contrast to the *tga2 tga5 tga6* mutant, growth phenotypes of the *tga1 tga4* mutant were identical to those of the wild type on control medium and on medium containing JA, OPDA, and PPA_1 (Fig. 1C). This shows that TGA1 and TGA4 are not involved in regulating root growth in response to oxylipins.

Regulation of phytoprostane-responsive genes is dependent on class II TGA factors but not on COI1 and MYC2

The results on COI1-dependent inhibition of root growth by phytoprostanes prompted the investigation of whether induction of phytoprostane-responsive genes is dependent on COI1. A limited analysis of this latter oxylipin response was previously documented in *coi1* mutant and wild-type plants using northern hybridization with two probes, one for the cytochrome P450 gene *CYP81D11*, which responds to diverse stimuli (Mueller *et al*., 2008; Matthes *et al*., 2010; Köster *et al*., 2012), and the other one for the OPDA reductase genes *OPR1/2*, which are phytoprostane responsive but also up-regulated after OPDA and JA treatment (Mueller *et al*., 2008). To challenge these previous findings, a more comprehensive analysis was performed using an independent method. Quantitative real-time PCR analysis of the above-mentioned genes as well as the glutathione *S*-transferase genes *GST6* and *GST25*, which are related to detoxification, and the *TolB*-like gene was performed; all three genes are phytoprostane responsive; *GST6* and *TolB*-like genes also show some up-regulation after OPDA treatment (Mueller *et al*., 2008). To discriminate the effects of different classes of oxylipins, the MYC2 transcription factor mutant *jin1* and expression of the vegetative storage protein gene *VSP1*, which is not responsive to phytoprostanes but shows COI1-dependent induction after JA treatment, were tested.

Relative to the wild type, induction of all tested phytoprostane-responsive genes by PPA_1 or OPDA was not reduced in the *jin1* and *coi1* mutants (Fig. 2). The trend of the previously reported reduced induction of *CYP81D11* in the *coi1* mutant by reactive oxylipins (Mueller *et al*., 2008) was confirmed; methodological differences are probably responsible for quantitative differences between northern hybridization and

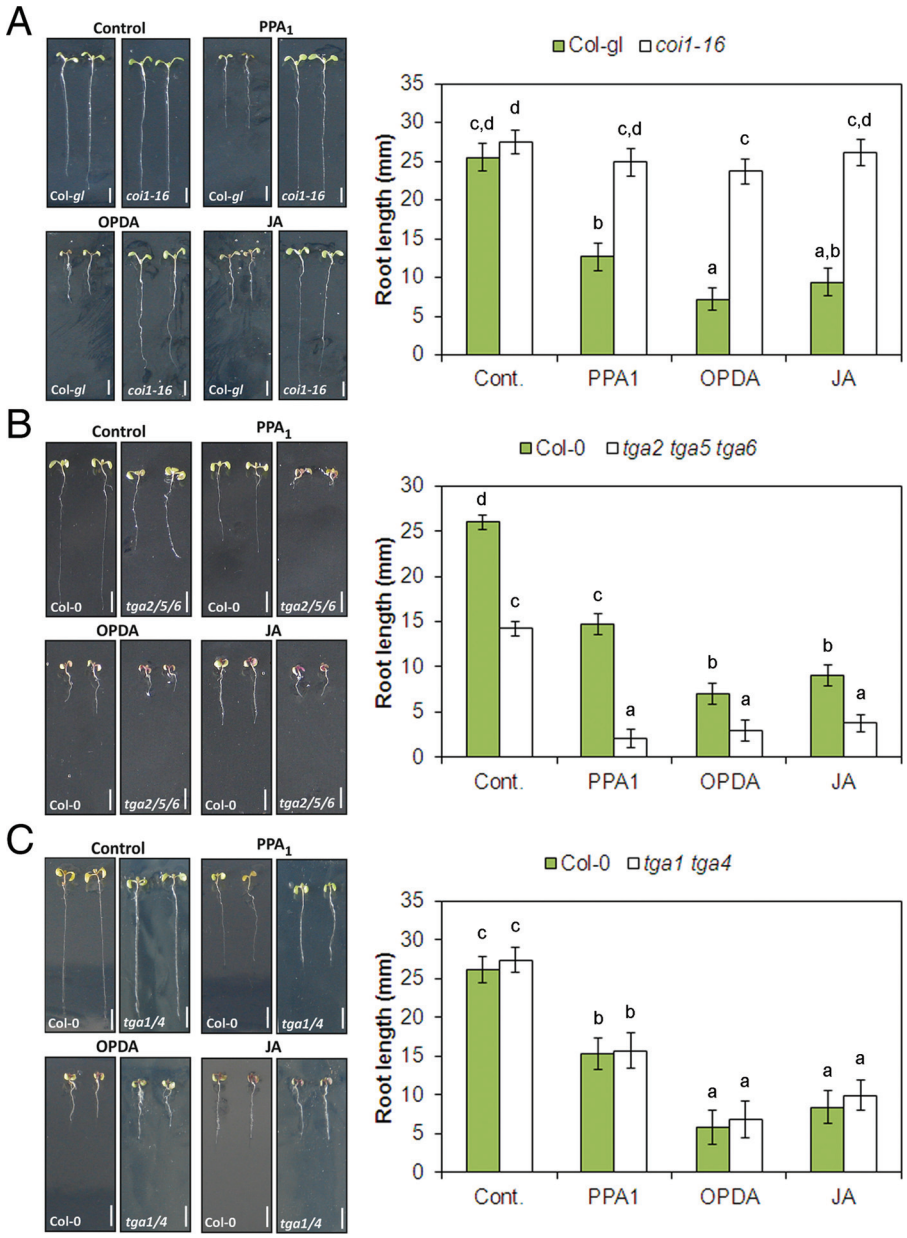

Fig. 1. Inhibition of root growth by oxylipins in different mutants. Seedlings of *coi1-16* (A), *tga2 tga5 tga6* (B), and *tga1 tga4* (C) were grown together with their corresponding wild types on vertically oriented MS agar plates containing phytoprostane A_1 (PPA_1), 12-oxo phytodienoic acid (OPDA), or jasmonic acid (JA) in a final concentration of 25 µM, or the solvent <2% methanol (control or Cont.). Root lengths were measured after 8 d of growth. Shown are means of 20 seedlings ±95% confidence intervals. Letters indicate significant differences among means. Independent experiments (six for *tga2 tga5 tga6*, four for *coi1* and *tga1 tga4*) were performed with similar results.

Table 1. Oxylipin-mediated root growth inhibition in the allene oxide synthase mutant *dde2* and wild-type (Col-0) *A. thaliana*.

	Col-0			***dde2***		
	Control	**25 µM JA**	**25 µM PPA_1**	**Control**	**25 µM JA**	**25 µM PPA_1**
Length (mm)	21.9 ± 1.8	6.7 ± 1.8	10.2 ± 1.7	24.4 ± 1.9	7.2 ± 1.7	12.2 ± 1.8
% Length	100	31	47	100	30	50

Sterilized seeds of Col-0 and *dde2-2* were grown on vertically oriented square Petri dishes containing MS medium supplemented with 2% (w/v) sucrose and oxylipins in a final concentration of 25 µM. Control treatments contained the solvent methanol (<2%). Root length was determined after 7 d. Shown are means ±95% confidence intervals of 14–16 seedlings. Mann–Whitney U-tests revealed no significant effect of genotypes on treatment ($P \leq 0.129$).

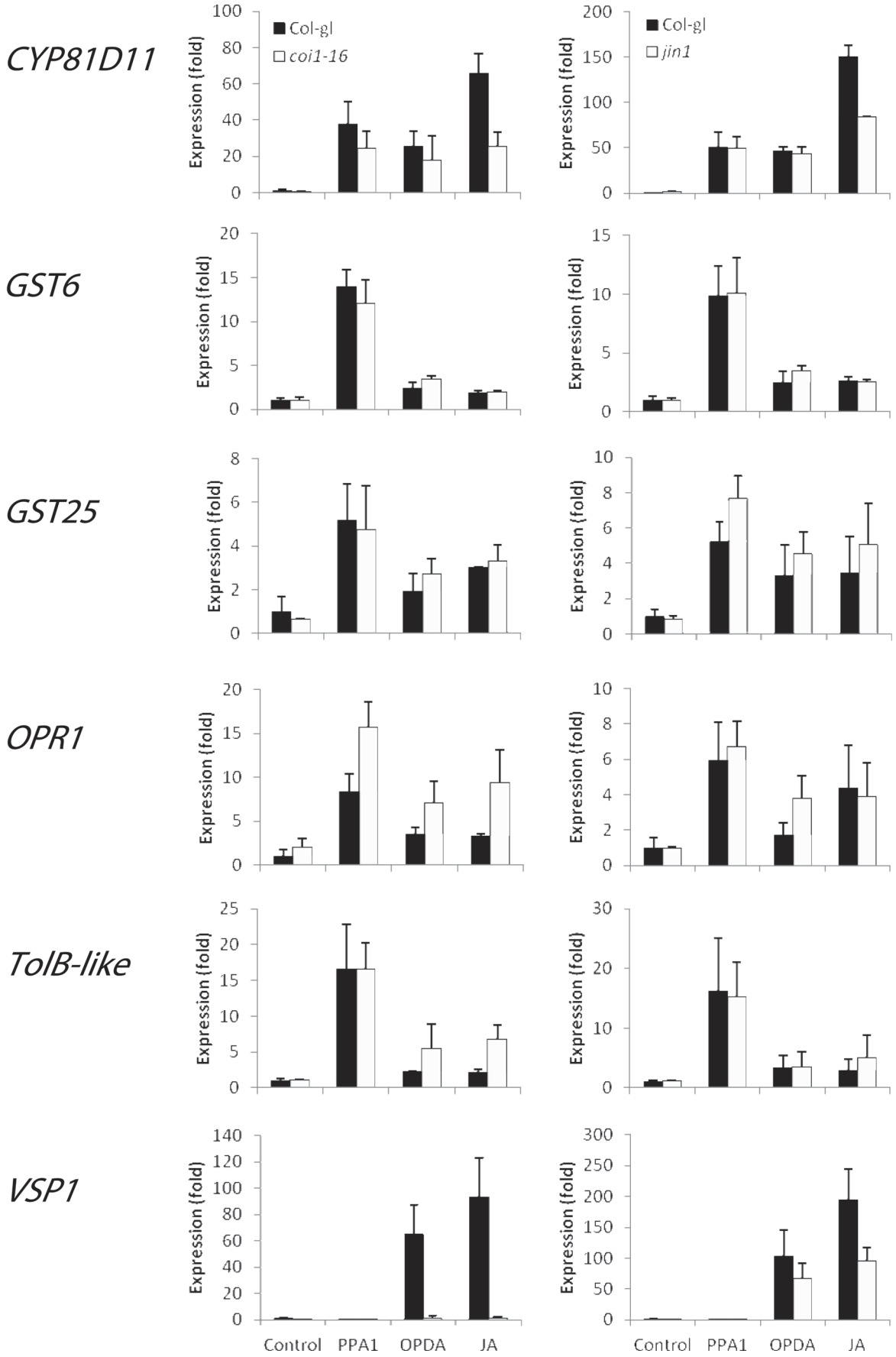

Fig. 2. Expression of oxylipin-responsive genes in the wild type and in mutants of the jasmonate pathway, *coi1* (left column) and *jin1* (right column). Seedlings were grown for 10 d in MS medium containing 2% sucrose under short-day conditions. The medium was

quantitative real-time PCR because *CYP81D11* belongs to a gene family with 15 members (Bak *et al.*, 2011). Up-regulation of *VSP1* and *CYP81D11* after JA treatment was clearly reduced in both mutants. Reduction of *VSP1* induction was stronger in the *coi1* mutant than in the *jin1* mutant, which is in agreement with published data (Benedetti *et al.*, 1995; Berger *et al.*, 1996). The *jin1* mutant has a small effect on *VSP1* expression because MYC2 acts in concert with MYC3 and MYC4 to regulate the expression of *VSP1* (Fernandez-Calvo *et al.*, 2011). Together, these data show that, in contrast to inhibition of root growth, induction of the tested phytoprostane-responsive genes is not dependent on COI1.

It was previously shown by microarray and northern analysis that induction of *CYP81D11* and *OPR1/2* genes by oxylipins is reduced in the *tga2 tga5 tga6* mutant (Mueller *et al.*, 2008). To compare the response of the triple mutant to exogenous JA and reactive oxylipins, target gene expression was analysed by quantitative reverse transcription PCR. To determine whether class II TGA factors specifically regulate oxylipin-induced gene expression, the class I TGA factor mutant *tga1 tga4* was tested.

The *tga2 tga5 tga6* mutant exhibited lower induction of *CYP81D11*, *GST25*, *OPR1*, and *TolB*-like by PPA_1 and OPDA in comparison with the wild type. Expression of *GST6* showed a tendency to lower induction than in the wild type, especially after treatment with OPDA (Fig. 3). These results are consistent with published data on *CYP81D11*, *OPR1*, *TolB*-like, and *GST6* expression (Mueller *et al.*, 2008). In addition, the induction of all tested genes by JA was lower relative to the wild type. This result confirms the previous conception that, besides their involvement in responses to OPDA and phytoprostanes, TGA2, TGA5, and TGA6 mediate responses to exogenous JA (Mueller *et al.*, 2008; Köster *et al.*, 2012). In contrast to the triple mutant, induction of all tested genes was not reduced in the *tga1 tga4* mutant. This suggests that TGA1 and TGA4 are not necessary for oxylipin responses.

Differential regulation of phytoprostane-responsive genes in tga6, tga2 tga5, *and* tga2 tga5 tga6 *mutants*

To test the individual contributions of TGA2, TGA5, and TGA6 to cyclopentenone-regulated *CYP81D11*, *OPR1*, and *GST25* expression, *tga6*, *tga2 tga5*, and *tga2 tga5 tga6* mutants were used. In addition to OPDA, *A. thaliana* seedlings grown in MS medium were challenged with PGA_1, a commercially available and structurally related cyclopentenone, which was previously shown to bind covalently to AtGST6 (Dueckershoff *et al.*, 2008).

CYP81D11 was induced 60- to 70-fold after treatment of wild-type seedlings for 4 h with OPDA or PGA_1 (Fig. 4). *CYP81D11* reached >70% of the wild-type induction level in the *tga6* mutant irrespective of the stimulus, suggesting that the absence of TGA6 does not have a significant effect on cyclopentenone-induced expression of this gene. Basal *CYP81D11* levels did not differ between the *tga6* mutant and the wild type, but basal expression levels were reduced >4-fold in the *tga2 tga5* and *tga2 tga5 tga6* mutants. Both OPDA- and PGA_1-stimulated expression of *CYP81D11* was significantly reduced in the *tga2 tga5* double mutant, reaching <20% of induced wild-type levels. A further reduction in oxylipin-induced *CYP81D11* expression occurred in the *tga2 tga5 tga6* mutant, reaching <3% of wild-type expression, which was not significantly different from uninduced wild-type levels. TGA6 therefore exerts a significant effect on *CYP81D11* expression in the absence but not in the presence of TGA2 and TGA5.

OPR1 expression increased 10- and 21-fold after treatment of wild-type seedlings with OPDA and PGA_1, respectively (Fig. 4). Basal *OPR1* levels did not vary much between mutant and wild-type seedlings. In the *tga6* mutant, expression of *OPR1* reached only 46% and 26% of wild-type levels after induction with OPDA and PGA_1, respectively. The response to PGA_1 was significantly reduced, indicating that TGA6 plays an essential role in *OPR1* induction. Up-regulation of *OPR1* by OPDA reached 26% of wild-type levels in the *tga2 tga5* mutant. Induction of *OPR1* by PGA_1 was significantly less in the *tga2 tga5* mutant, reaching only 10% of wild-type levels. OPDA- and PGA_1-responsive expression of *OPR1* was further decreased in the *tga2 tga5 tga6* mutant.

GST25 was induced 16- and 5-fold after treatment of wild-type plants with OPDA and PGA_1, respectively (Fig. 4). *GST25* expression reached 57% and 45% of wild-type levels in the *tga6* mutant after induction with OPDA and PGA_1, respectively. Cyclopentenone-induced *GST25* expression levels were very similar in the *tga6* and *tga2 tga5* mutant, suggesting that induced *GST25* expression is regulated similarly by TGA2 and TGA5 and by TGA6. The induction level in the *tga2 tga5 tga6* mutant was <3% relative to the wild type and did not differ from uninduced wild-type levels. Quantitative differences in *GST25* or *OPR1* induction levels among experiments (as compared with Figs 2 and 3) are probably attributable to subtle changes in plant growth conditions.

Separate effects of three TGA factors on OPDA-induced gene expression

To examine further the contribution of individual TGA factors to OPDA-induced gene expression, *TGA2*-, *TGA5*-, or *TGA6*-overexpressing *A. thaliana* lines (Zander *et al.*, 2010)

exchanged for 75 µM phytoprostane A_1 (PPA_1), 75 µM 12-oxo phytodienoic acid (OPDA), 75 µM jasmonic acid (JA), or the solvent 0.5% methanol (control). After a treatment for 4 h, RNA was extracted, converted into cDNA, and amplified using quantitative reverse transcription PCR. Expression of the cytochrome P450 gene *CYP81D11*, the glutathione *S*-transferase genes *GST6* and *GST25*, the OPDA reductase gene *OPR1*, the *TolB*-like gene, and the gene encoding vegetative storage protein1, *VSP1*, is shown. Expression was normalized to the actin gene *Act2/8*, which was used as a constitutively expressed internal control. Expression of the wild-type control treatment was set to 1 and all other data were expressed relative to it. Presented are means and standard deviations of three independent experiments with different biological replicates.

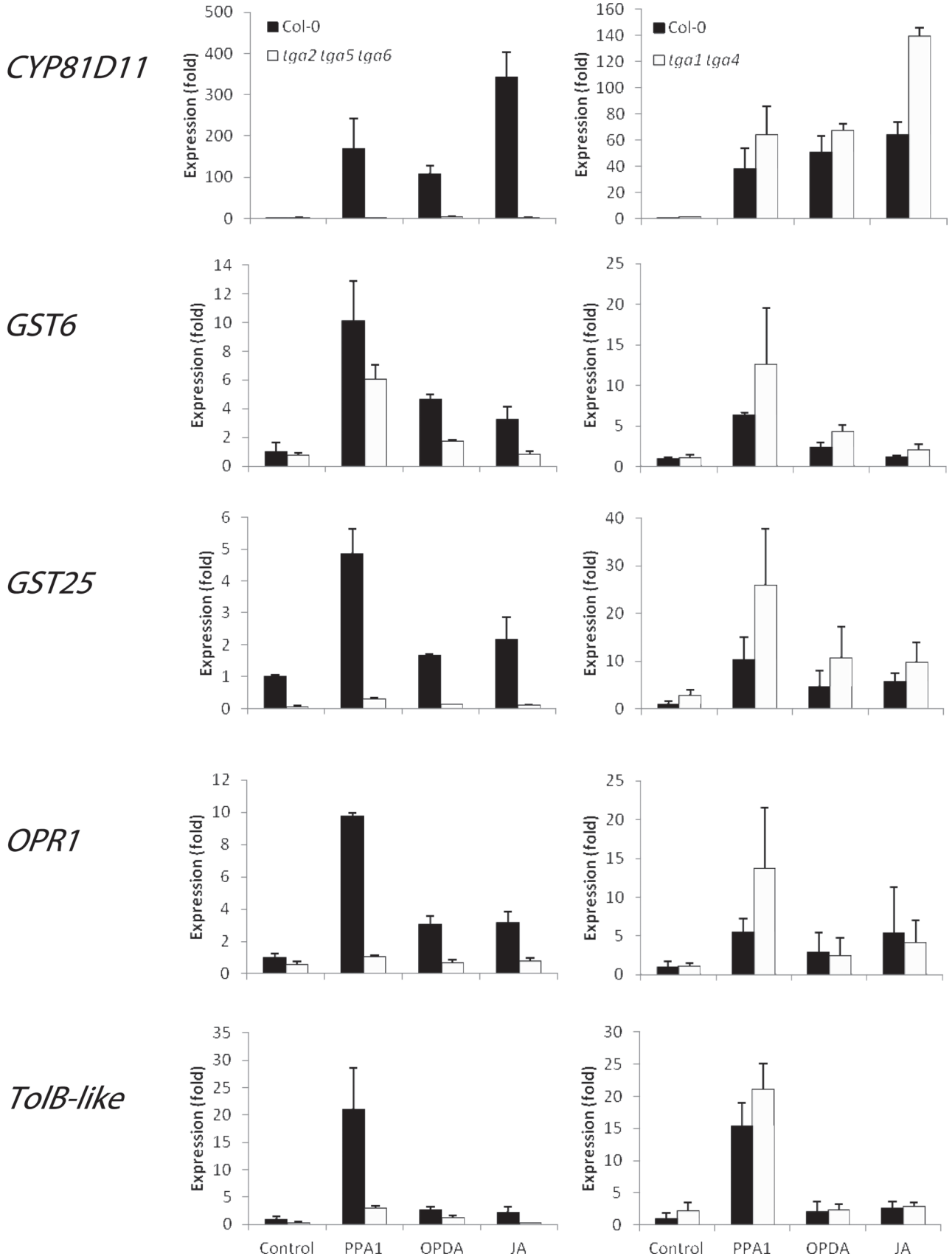

Fig. 3. Expression of oxylipin-responsive genes in the wild type and in *tga2 tga5 tga6* (left column) and *tga1 tga4* mutants (right column). Seedlings were grown for 10 d in MS medium containing 2% sucrose under short-day conditions. The medium was exchanged for 75 μM phytoprostane A_1 (PPA_1), 75 μM 12-oxo phytodienoic acid (OPDA), 75 μM jasmonic acid (JA), or the solvent 0.5% methanol (control). After a treatment for 4 h, RNA was extracted, converted into cDNA, and amplified using quantitative reverse

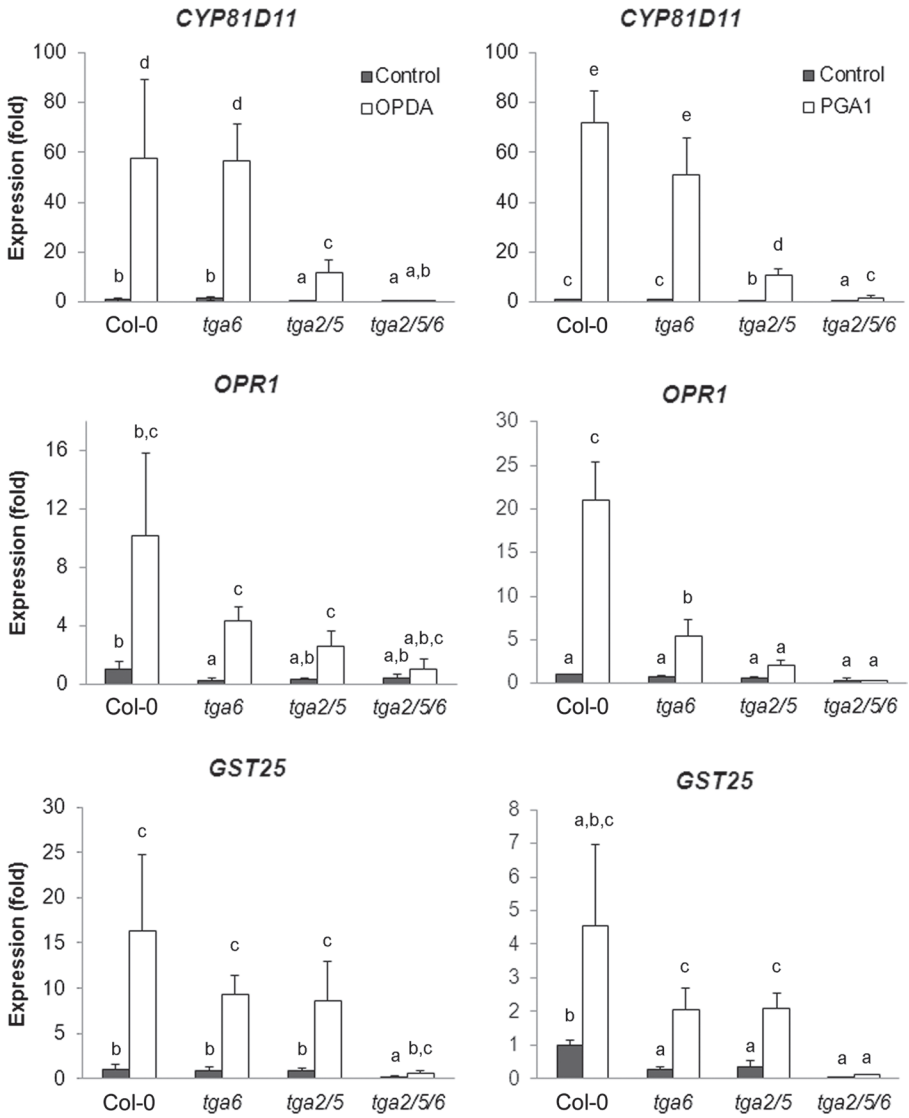

Fig. 4. Expression of oxylipin-responsive genes in the wild type and *tga* mutants. Seedlings were grown for 10 d in MS medium containing 1% sucrose under short-day conditions. The medium was exchanged for 75 µM 12-oxo phytodienoic acid (OPDA), 75 µM prostaglandin A_1 (PGA_1), or the solvent 0.5% methanol (control). After a treatment for 4 h, RNA was extracted, converted into cDNA, and amplified using quantitative reverse transcription PCR. Expression of the cytochrome P450 gene *CYP81D11*, the OPDA reductase gene *OPR1*, and the glutathione *S*-transferase gene *GST25* is shown. Expression was normalized to the actin gene *Act2/8*, which was used as a constitutively expressed internal control. Expression of the wild-type control treatment was set to 1 and all other data were expressed relative to it. Means and standard errors of three biological replicates are shown. Significant differences among means indicated by letters were determined using the Relative Expression Software Tool V2.0.13 (Qiagen, Hilden, Germany).

were used. TGA protein expression was readily detected in crude extracts from overexpressing plants (Supplementary Fig. S1 at *JXB* online). TGA protein expression varied among overexpressing lines but did not substantially alter the induction of target gene expression (Supplementary Figs S2, S3).

OPDA treatment of wild-type seedlings increased *CYP81D11* expression 93-fold (Fig. 5). This level of induction was consistent across experiments in the wild-type background Col-0 (Figs 3, 4), but induction of *CYP81D11* appeared to be quantitatively lower in the genotype Col-*gl* (Fig. 2). No induction of *CYP81D11* by OPDA was observed in the *tga2 tga5 tga6* mutant, which served as the genetic background for all three lines overexpressing TGA factors. *CYP81D11* expression was significantly increased after OPDA treatment of

transcription PCR. Expression of the cytochrome P450 gene *CYP81D11*, the glutathione *S*-transferase genes *GST6* and *GST25*, the OPDA reductase gene *OPR1*, and the *TolB*-like gene is shown. Expression was normalized to the actin gene *Act2/8*, which was used as a constitutively expressed internal control. Expression of the wild-type control treatment was set to 1 and all other data were expressed relative to it. Presented are means and standard deviations of three independent experiments with different biological replicates.

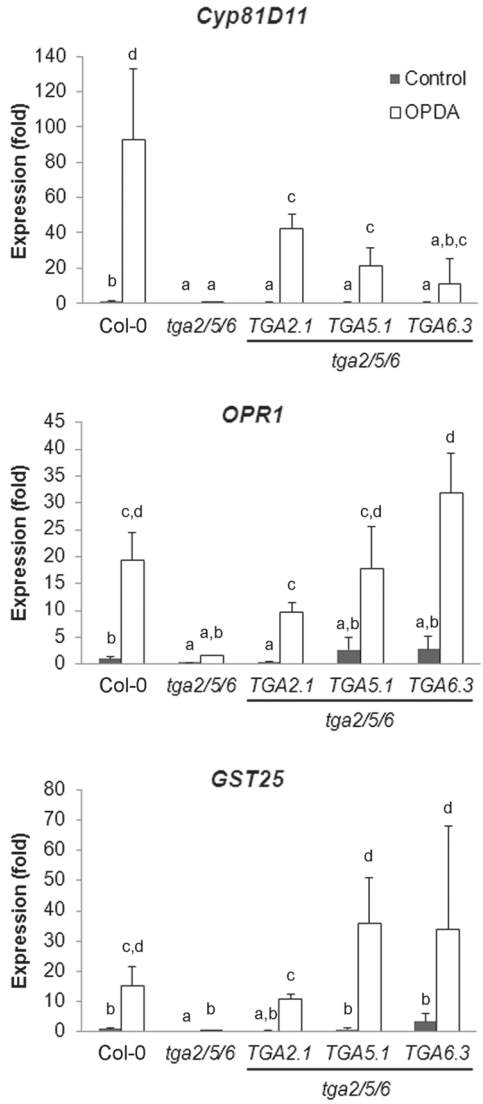

Fig. 5. Expression of oxylipin-responsive genes in wild-type, *tga2 tga5 tga6* mutant, and *TGA*-overexpressing plants. *TGA* overexpression occurred in the background of the *tga2 tga5 tga6* mutant. Seedlings were grown for 10 d in MS medium containing 1% sucrose under short-day conditions. The medium was exchanged for 75 μM 12-oxo phytodienoic acid (OPDA) or the solvent 0.5% methanol (control). After a treatment for 4 h, RNA was extracted, converted into cDNA, and amplified using quantitative reverse transcription PCR. Expression of the cytochrome P450 gene *CYP81D11*, the OPDA reductase gene *OPR1*, and the glutathione *S*-transferase gene *GST25* is shown. Expression was normalized to the actin gene *Act2/8*, which was used as a constitutively expressed internal control. Expression of the wild type control treatment was set to 1 and all other data were expressed relative to it. Means and standard errors of three biological replicates are shown. Significant differences among means indicated by letters were determined using the Relative Expression Software Tool V2.0.13 (Qiagen, Hilden, Germany).

TGA2.1- and *TGA5.1*-overexpressing lines by 46% and 23% of wild-type levels, respectively. However, OPDA induction of *CYP81D11* was not significant in the *TGA6.3*-overexpressing line, reaching only 12% of wild-type levels. These results support the *tga* mutant data (Fig. 4) and demonstrate that TGA6 is not sufficient for induced *CYP81D11* expression.

Effects of *TGA2.1*, *TGA5.1* and *TGA6.3* overexpression on OPDA-induced expression of *OPR1* and *GST25* were similar and were distinct from those of *CYP81D11*. Overexpression of each of the three transcription factors overcame the lack of *OPR1* and *GST25* induction after OPDA treatment in the *tga2 tga5 tga6* mutant. Although TGA2 made a significant contribution to OPDA-induced expression of *OPR1* and *GST25*, the effects of TGA5 and TGA6 were quantitatively larger.

Based on data from both mutant and transgenic seedlings, the response of *CYP81D11* to OPDA is regulated directly or indirectly by TGA2 and TGA5. In contrast, TGA5 and TGA6 make a quantitatively larger contribution to OPDA-induced expression of *OPR1* and *GST25* than TGA2. These data suggest that at least two classes of OPDA-regulated genes exist.

Discussion

COI1 mediates root growth inhibition in response to phytoprostanes independent of jasmonates

Whereas root growth was not inhibited by JA, OPDA, or PPA_1 in the *coi1* mutant (Fig. 1A), the *AOS* mutant *dde2* was fully sensitive to phytoprostane treatment (Table 1). This finding illustrates that root growth in this JA- and OPDA-deficient mutant is dependent on COI1 and that COI1 mediates jasmonate-independent responses to an electrophilic oxylipin. While similar JA-Ile-independent COI1-mediated responses were previously documented (Ribot *et al.*, 2008; Adams and Turner, 2010; Stotz *et al.*, 2011; Köster *et al.*, 2012; Ralhan *et al.*, 2012), the underlying mechanism has not been resolved. Based on these published results, apparently two jasmonate-independent COI1 pathways exist. Unlike the *opr3* mutant, *aos* and *coi1* mutants are impaired in defence responses against the necrotrophic ascomycete *Sclerotinia sclerotiorum* (Stotz *et al.*, 2011) and during wound-induced expression of *AtPHO1;H10* (Ribot *et al.*, 2008), suggesting that OPDA mediates JA-Ile-independent COI1 responses. On the other hand, ethylene-dependent inhibition of root growth (Adams and Turner, 2010), susceptibility to *Verticillium longisporum* (Ralhan *et al.*, 2012), and induction of *CYP81D11* in response to xenobiotics (Köster *et al.*, 2012) are altered in the *coi1* but not in the *aos* mutant, suggesting that in this case COI1 exerts its effects independently of OPDA. Elegant grafting experiments showed that susceptibility to *V. longisporum* is dependent on a COI1-specific recognition event in the root (Ralhan *et al.*, 2012), suggesting that this organ may also play a role in mediating oxylipin responses. In analogy, we now show that the phytoprostane PPA_1 signals through COI1 independently of OPDA and JA biosynthesis.

COI1 interacts with JAZ1, JAZ3, JAZ6, JAZ9, and JAZ10 in a JA-Ile- and coronatine-dependent manner (Melotto *et al.*, 2008; Chung and Howe, 2009; Sheard *et al.*, 2010). Although OPDA does not facilitate interactions of COI1

with JAZ1, JAZ3, and JAZ9 (Melotto *et al*., 2008; Chung and Howe, 2009), the possibility cannot be excluded that cyclopentenones may promote interactions between COI1 and other JAZ proteins. JA-Ile induces 10 of the 12 JAZ family members as part of a negative feedback loop (Chini *et al*., 2007). Analysis of transcript profiling in response to the phytoprostane PPA_1 (Mueller *et al*., 2008) did not indicate regulation of JAZ genes by this compound. Alternatively, binding of phytoprostanes to COI1 may facilitate interactions with other proteins that are not related to JAZ proteins but nevertheless act as co-receptors of COI1.

TGA factors 2, 5, and 6 activate oxylipin-responsive gene expression but impede inhibition of root growth by oxylipins

The TGA factors 2, 5, and 6 were shown to act as redundant members of the class II TGA factors during the establishment of systemic acquired resistance, which is regulated by the salicylic acid (SA) pathway (Zhang *et al*., 2003). In addition, these transcription factors are involved in regulating gene expression in response to the jasmonate/ethylene pathway (Zander *et al*., 2010). This pathway is important for resistance to necrotrophic pathogens, and the *tga2 tga5 tga6* mutant is more susceptible to *Botrytis cinerea* than wild-type plants (Zander *et al*., 2010). A possible explanation for this hypersusceptibility is perhaps reduced jasmonate/ethylene signalling and a strongly reduced expression of genes related to detoxification (Mueller *et al*., 2008), leading to a reduced and slower metabolism of phytoprostanes and other toxic compounds. This is supported by results showing that in the *tga2 tga5 tga6* mutant, cell death is elevated after treatment with tert-butyl hydroperoxide (Supplementary Fig. S4 at *JXB* online) and that sensitivity to xenobiotics is increased relative to the wild type (Fode *et al*., 2008). Collectively, these data suggest that these three TGA factors play an important role in detoxification responses of plants.

The fact that the *tga2 tga5 tga6* mutant still responded to oxylipins with a reduction in root growth (Fig. 1) suggests that this response is not dependent on these transcription factors. Although the growth of the triple mutant was reduced on MS agar medium relative to the wild type, inhibition of root growth by PPA_1 was quantitatively larger in the *tga2 tga5 tga6* mutant than in the wild type. The hypersensitivity of the triple mutant to a phytoprostane seems to support the proposed antagonism between these three TGA factors and MYC2 affecting ORA59 expression and jasmonate/ethylene-related gene expression (Zander *et al*., 2010).

TGA-specific regulation of phytoprostane-responsive target genes

The putative detoxification genes *CYP81D11*, *OPR1*, and *GST25* responded differently to TGA2, TGA5, and TGA6. *CYP81D11* differed from *GST25* and *OPR1* in the level of induction by cyclopentenones but also in the specificity of induction by different TGA factors. Cyclopentenone-induced expression of *CYP81D11* was more strongly regulated by TGA2 and TGA5 than by TGA6 (Figs 4, 5). At the most, overexpression of TGA factors resulted in an OPDA induction of ~50% relative to wild-type levels (Fig. 5). Thus, overexpression of single TGA factors results in partial induction of *CYP81D11* expression, raising the possibility that TGA factors may become limiting due to the heterodimerization requirements of these transcription factors. In contrast, overexpression of TGA5 or TGA6 in the background of the *tga2 tga5 tga6* mutant resulted in wild-type levels of *GST25* and *OPR1* expression after OPDA treatment (Fig. 5), suggesting that individual TGA factors can be sufficient for the induction of these genes. These results show that control of gene expression by TGA factors varies among target genes. In contrast to the results presented here, SA-induced expression of *PR1* is blocked in the *tga2 tga5 tga6* mutant, but wild-type induction levels are reached in *tga6* and *tga2 tga5* mutants, which demonstrates transcription factor redundancy with respect to *PR1* expression (Zhang *et al*., 2003). On the other hand, expression of *PDF1.2* after induction with methyl jasmonate and 1-aminocyclopropane-1-carboxylic acid is similar in wild-type and *tga6* mutant plants, whereas stimulus-induced expression is equally low in *tga2 tga5* and *tga2 tga5 tga6* mutants (Zander *et al*., 2010). Thus, expression of *PDF1.2* under these conditions is strictly dependent on TGA2 and TGA5. However, TGA factors indirectly regulate *PDF1.2* expression (Zander *et al*., 2010).

Unlike *GST25*, which is exclusively regulated by TGA2, TGA5, and TGA6, *CYP81D11* was recently shown to be co-regulated by these TGA factors and COI1 (Köster *et al*., 2012). Sequence analysis of the *OPR1* promoter provides no evidence for the presence of a MYC2-responsive G-box, also suggesting a fundamental difference in regulation of *CYP81D11* versus *GST25* and *OPR1* genes.

Contrast of the responses to COI1 or TGA2, TGA5, and TGA6

COI1 as well as TGA2, TGA5, and TGA6 induce related but distinct defence responses. For instance, susceptibilities of both *coi1* and *tga2 tga5 tga6* mutants to *B. cinerea* are elevated relative to the wild type (Thomma *et al*., 1998; Zander *et al*., 2010). Likewise, induction of *PDF1.2* expression after *B. cinerea* inoculation is severely reduced in both types of mutants (Guo and Stotz, 2007; Zander *et al*., 2010). However, *coi1* and *tga2 tga5 tga6* mutants differ in *cis*-jasmone-responsive gene expression patterns (Matthes *et al*., 2010), demonstrating clear differences in these signal transduction pathways. This is not surprising because class II TGA factors were shown to activate indirectly the jasmonate/ethylene pathway that is controlled by COI1 (Zander *et al*., 2010). Given that COI1 also fulfils distinct roles in regulation of responses to JA and to pathogens via combinatorial jasmonate/ethylene signalling, differences in observed physiological (Fig. 1) and defence responses (Figs 2, 3) can be reconciled.

Whereas PPA_1 activates the expression of stress and detoxification genes, this compound down-regulates the expression of genes that contribute to cell growth and division (Mueller *et al*., 2008), which may explain the fact that roots respond

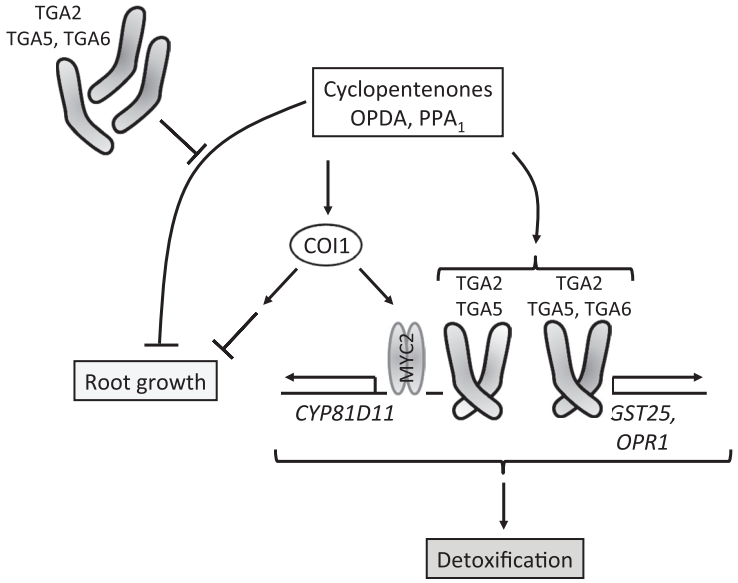

Fig. 6. Tentative model explaining the observed effects of cyclopentenone oxylipins on root growth and expression of detoxification genes. Roles of the jasmonate receptor COI1 and TGA transcription factors in mediating oxylipin signalling are highlighted. Individual contributions of TGA factors were determined for induction of detoxification genes but not for root growth inhibition. The *CYP81D11* promoter is primarily regulated by TGA2 and TGA5. *GST25* and *OPR1* promoters are primarily regulated by TGA5 and TGA6, although TGA2 does also contribute to the expression of these genes. MYC2 was previously shown to activate *CYP81D11* expression (Köster *et al*., 2012) presumably by binding to a G-box in the promoter sequence.

to phytoprostanes with growth inhibition (Fig. 1). Moreover, root growth inhibition in response to phytoprostanes is lessened by TGA2, TGA5, and TGA6, possibly because these proteins may influence the repression of gene expression associated with growth and division. In contrast, COI1 exerts a negative effect on root growth in response to cyclopentenones, although this receptor is only known to bind JA-Ile and coronatine.

Collectively, these data strongly suggest the existence of two phytoprostane signalling pathways (Fig. 6). One pathway regulates the expression of detoxification genes and is influenced positively by both COI1 and class II TGA factors. The second pathway inhibits root growth, which is mediated by COI1 but negatively influenced by the TGA factors. This proposed model can be reconciled with a previously published model on the antagonism between class II TGA factors and MYC2 (Zander *et al*., 2010).

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. Relative expression of TGA factors in overexpressing *A. thaliana* lines.

Figure S2. Oxylipin-responsive gene expression in wild-type, *tga2 tga5 tga6* mutant, and independent TGA-overexpressing plants.

Figure S3. Effect of different levels of TGA6 protein expression on plant growth and oxylipin-responsive gene expression.

Fig. S4. Cell death in *tga2 tga5 tga6* mutant and Col-0 (wild-type) seedlings.

Table S1. Primers and probes used for quantitative RT–PCR.

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References

Adams E, Turner J. 2010. COI1, a jasmonate receptor, is involved in ethylene-induced inhibition of Arabidopsis root growth in the light. *Journal of Experimental Botany* **61,** 4373–4386.

Bak S, Beisson F, Bishop G, Hamberger B, Hofer R, Paquette S, Werck-Reichhart D. 2011. Cytochromes p450. *The Arabidopsis Book* **9,** e0144.

Benedetti CE, Xie DX, Turner JG. 1995. COI1-dependent expression of an Arabidopsis vegetative storage protein in flowers and siliques and in response to coronatine or methyl jasmonate. *Plant Physiology* **109,** 567–572.

Berger S, Bell E, Mullet JE. 1996. Two methyl jasmonate-insensitive mutants show altered expression of atvsp in response to methyl jasmonate and wounding. *Plant Physiology* **111,** 525–531.

Chini A, Fonseca S, Fernandez G, *et al.* 2007. The JAZ family of repressors is the missing link in jasmonate signalling. *Nature* **448,** 666–673.

Chung HS, Howe GA. 2009. A critical role for the TIFY motif in repression of jasmonate signaling by a stabilized splice variant of the JASMONATE ZIM-domain protein JAZ10 in Arabidopsis. *The Plant Cell* **21,** 131–145.

Dave A, Hernandez ML, He Z, Andriotis VM, Vaistij FE, Larson TR, Graham IA. 2011. 12-Oxo-phytodienoic acid accumulation during seed development represses seed germination in Arabidopsis. *The Plant Cell* **23,** 583–599.

Dave A, Graham IA. 2012. Oxylipin signaling: a distinct role for the jasmonic acid precursor *cis*-(+)-12-oxo-phytodienoic acid (*cis*-OPDA). *Frontiers in Plant Science* **3,** 42.

Devoto A, Ellis C, Magusin A, Chang HS, Chilcott C, Zhu T, Turner JG. 2005. Expression profiling reveals COI1 to be a key regulator of genes involved in wound- and methyl jasmonate-induced secondary metabolism, defence, and hormone interactions. *Plant Molecular Biology* **58,** 497–513.

Dueckershoff K, Mueller S, Mueller MJ, Reinders J. 2008. Impact of cyclopentenone-oxylipins on the proteome of *Arabidopsis thaliana*. *Biochimica et Biophysica Acta* **1784,** 1975–1985.

Ellinger D, Stingl N, Kubigsteltig, II, Bals T, Juenger M, Pollmann S, Berger S, Schuenemann D, Mueller MJ. 2010. DONGLE and DEFECTIVE IN ANTHER DEHISCENCE1 lipases are not essential for wound- and pathogen-induced jasmonate biosynthesis: redundant lipases contribute to jasmonate formation. *Plant Physiology* **153,** 114–127.

Ellis C, Turner JG. 2002. A conditionally fertile *coi1* allele indicates cross-talk between plant hormone signalling pathways in *Arabidopsis thaliana* seeds and young seedlings. *Planta* **215,** 549–556.

Farmer EE, Davoine C. 2007. Reactive electrophile species. *Current Opinion in Plant Biology* **10,** 380–386.

Fernandez-Calvo P, Chini A, Fernandez-Barbero G, *et al.* 2011. The Arabidopsis bHLH transcription factors MYC3 and MYC4 are targets of JAZ repressors and act additively with MYC2 in the activation of jasmonate responses. *The Plant Cell* **23,** 701–715.

Fode B, Siemsen T, Thurow C, Weigel R, Gatz C. 2008. The Arabidopsis GRAS protein SCL14 interacts with class II TGA transcription factors and is essential for the activation of stress-inducible promoters. *The Plant Cell* **20,** 3122–3135.

Guo X, Stotz HU. 2007. Defense against *Sclerotinia sclerotiorum* in Arabidopsis is dependent on jasmonic acid, salicylic acid, and ethylene signaling. *Molecular Plant-Microbe Interactions* **20,** 1384–1395.

Howe GA, Lightner J, Browse J, Ryan CA. 1996. An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. *The Plant Cell* **8,** 2067–2077.

Katsir L, Schilmiller AL, Staswick PE, He SY, Howe GA. 2008. COI1 is a critical component of a receptor for jasmonate and the bacterial virulence factor coronatine. *Proceedings of the National Academy of Sciences, USA* **105,** 7100–7105.

Kesarwani M, Yoo J, Dong X. 2007. Genetic interactions of TGA transcription factors in the regulation of pathogenesis-related genes and disease resistance in Arabidopsis. *Plant Physiology* **144,** 336–346.

Köster J, Thurow C, Kruse K, Meier A, Iven T, Feussner I, Gatz C. 2012. Xenobiotic- and jasmonic acid-inducible signal transduction pathways have become interdependent at the Arabidopsis *CYP81D11* promoter. *Plant Physiology* **159,** 391–402.

Laurie-Berry N, Joardar V, Street IH, Kunkel BN. 2006. The *Arabidopsis thaliana JASMONATE INSENSITIVE 1* gene is required for suppression of salicylic acid-dependent defenses during infection by *Pseudomonas syringae*. *Molecular Plant-Microbe Interactions* **19,** 789–800.

Lorenzo O, Chico JM, Sanchez-Serrano JJ, Solano R. 2004. JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in *Arabidopsis*. *The Plant Cell* **16,** 1938–1950.

Malek Bv, Graaff Evd, Schneitz K, Keller B. 2002. The *Arabidopsis* male-sterile mutant *dde2-2* is defective in the allene oxide synthase gene encoding one of the key enzymes of the jasmonic acid biosynthesis pathway. *Planta* **216,** 187–192.

Matthes MC, Bruce TJ, Ton J, Verrier PJ, Pickett JA, Napier JA. 2010. The transcriptome of cis-jasmone-induced resistance in *Arabidopsis thaliana* and its role in indirect defence. *Planta* **232,** 1163–1180.

McConn M, Browse J. 1996. The critical requirement for linolenic acid is pollen development, not photosynthesis, in an Arabidopsis mutant. *The Plant Cell* **8,** 403–416.

McConn M, Creelman RA, Bell E, Mullet JE, Browse J. 1997. Jasmonate is essential for insect defense in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* **94,** 5473–5477.

Melotto M, Mecey C, Niu Y, *et al.* 2008. A critical role of two positively charged amino acids in the Jas motif of Arabidopsis JAZ proteins in mediating coronatine- and jasmonoyl isoleucine-dependent interactions with the COI1 F-box protein. *The Plant Journal* **55,** 979–988.

Mene-Saffrane L, Dubugnon L, Chetelat A, Stolz S, Gouhier-Darimont C, Farmer EE. 2009. Nonenzymatic oxidation of trienoic fatty acids contributes to reactive oxygen species management in Arabidopsis. *Journal of Biological Chemistry* **284,** 1702–1708.

Mueller MJ. 2004. Archetype signals in plants: the phytoprostanes. *Current Opinion in Plant Biology* **7,** 441–448.

Mueller S, Hilbert B, Dueckershoff K, Roitsch T, Krischke M, Mueller MJ, Berger S. 2008. General detoxification and stress responses are mediated by oxidized lipids through TGA transcription factors in Arabidopsis. *The Plant Cell* **20,** 768–785.

Nickstadt A, Thomma BPHJ, Feussner I, Kangasjarvi J, Zeier J, Loeffler C, Scheel D, Berger S. 2004. The jasmonate-insensitive mutant *jin1* shows increased resistance to biotrophic as well as necrotrophic pathogens. *Molecular Plant Pathology* **5,** 425–434.

Parchmann S, Gundlach H, Mueller MJ. 1997. Induction of 12-oxo-phytodienoic acid in wounded plants and elicited plant cell cultures. *Plant Physiology* **115,** 1057–1064.

Park JH, Halitschke R, Kim HB, Baldwin IT, Feldmann KA, Feyereisen R. 2002. A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in Arabidopsis due to a block in jasmonic acid biosynthesis. *The Plant Journal* **31,** 1–12.

Pieterse CM, van der Does D, Zamioudis C, Leon-Reyes A, van Wees SC. 2012. Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology* **28,** 28.21–28.33.

Raacke I, Mueller MJ, Berger S. 2006. Defects in allene oxide synthase and 12-oxo-phytodienoic acid reductase alter the resistance to *Pseudomonas syringae* and *Botrytis cinerea*. *Journal of Phytopathology* **154,** 740–744.

Ralhan A, Schottle S, Thurow C, Iven T, Feussner I, Polle A, Gatz C. 2012. The vascular pathogen *Verticillium longisporum* requires a jasmonic acid-independent COI1 function in roots to elicit disease symptoms in Arabidopsis shoots. *Plant Physiology* **159,** 1192–1203.

Ribot C, Zimmerli C, Farmer EE, Reymond P, Poirier Y. 2008. Induction of the Arabidopsis PHO1;H10 gene by 12-oxo-phytodienoic acid but not jasmonic acid via a CORONATINE INSENSITIVE1-dependent pathway. *Plant Physiology* **147,** 696–706.

Sanders PM, Lee PY, Biesgen C, Boone JD, Beals TP, Weiler EW, Goldberg RB. 2000. The *Arabidopsis DELAYED DEHISCENCE1* gene encodes an enzyme in the jasmonic acid synthesis pathway. *The Plant Cell* **12,** 1041–1061.

Sheard LB, Tan X, Mao H, *et al.* 2010. Jasmonate perception by inositol-phosphate-potentiated COI1–JAZ co-receptor. *Nature* **468,** 400–405.

Stintzi A, Browse J. 2000. The Arabidopsis male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. *Proceedings of the National Academy of Sciences, USA* **97,** 10625–10630.

Stotz HU, Jikumaru Y, Shimada Y, Sasaki E, Stingl N, Mueller MJ, Kamiya Y. 2011. Jasmonate-dependent and COI1-independent defense responses against *Sclerotinia sclerotiorum* in *Arabidopsis thaliana*: auxin is part of COI1-independent defense signaling. *Plant and Cell Physiology* **52,** 1941–1956.

Swiatek A, Lenjou M, Van Bockstaele D, Inze D, Van Onckelen H. 2002. Differential effect of jasmonic acid and abscisic acid on cell cycle progression in tobacco BY-2 cells. *Plant Physiology* **128,** 201–211.

Taki N, Sasaki-Sekimoto Y, Obayashi T, *et al.* 2005. 12-Oxo-phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in *Arabidopsis*. *Plant Physiology* **139,** 1268–1283.

Thines B, Katsir L, Melotto M, *et al.* 2007. JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling. *Nature* **448,** 661–665.

Thoma I, Loeffler C, Sinha AK, Gupta M, Krischke M, Steffan B, Roitsch T, Mueller MJ. 2003. Cyclopentenone isoprostanes induced by reactive oxygen species trigger defense gene activation and phytoalexin accumulation in plants. *The Plant Journal* **34,** 363–375.

Thomma BPHJ, Eggermont K, Penninckx IAMA, Mauch-Mani B, Vogelsang R, Cammue BPA, Broekaert WF. 1998. Separate jasmonate-dependent and salicylate-dependent defense-response pathways in Arabidopsis are essential for resistance to distinct microbial pathogens. *Proceedings of the National Academy of Sciences, USA* **95,** 15107–15111.

Ton J, Van Pelt JA, Van Loon LC, Pieterse CM. 2002. Differential effectiveness of salicylate-dependent and jasmonate/ethylene-dependent induced resistance in Arabidopsis. *Molecular Plant-Microbe Interactions* **15,** 27–34.

Wasternack C. 2007. Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. *Annals of Botany* **100,** 681–697.

Zander M, La Camera S, Lamotte O, Metraux JP, Gatz C. 2010. *Arabidopsis thaliana* class-II TGA transcription factors are essential activators of jasmonic acid/ethylene-induced defense responses. *The Plant Journal* **61,** 200–210.

Zhang Y, Tessaro MJ, Lassner M, Li X. 2003. Knockout analysis of Arabidopsis transcription factors TGA2, TGA5, and TGA6 reveals their redundant and essential roles in systemic acquired resistance. *The Plant Cell* **15,** 2647–2653.