Induction of the Arabidopsis *PHO1;H10* Gene by 12-Oxo-Phytodienoic Acid But Not Jasmonic Acid via a CORONATINE INSENSITIVE1-Dependent Pathway[1]

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Expression of *AtPHO1;H10*, a member of the Arabidopsis (*Arabidopsis thaliana*) *PHO1* gene family, is strongly induced following numerous abiotic and biotic stresses, including wounding, dehydration, cold, salt, and pathogen attack. *AtPHO1;H10* expression by wounding was localized to the cells in the close vicinity of the wound site. *AtPHO1;H10* expression was increased by application of the jasmonic acid (JA) precursor 12-oxo-phytodienoic acid (OPDA), but not by JA or coronatine. Surprisingly, induction of *AtPHO1;H10* by OPDA was dependent on the presence of CORONATINE INSENSITIVE1 (COI1). The induction of *AtPHO1;H10* expression by wounding and dehydration was dependent on COI1 and was comparable in both the wild type and the OPDA reductase 3-deficient (*opr3*) mutant. In contrast, induction of *AtPHO1;H10* expression by exogenous abscisic acid (ABA) was independent of the presence of either OPDA or COI1, but was strongly decreased in the ABA-insensitive mutant *abi1-1*. The involvement of the ABA pathway in regulating *AtPHO1;H10* was distinct between wounding and dehydration, with induction of *AtPHO1;H10* by wounding being comparable to wild type in the ABA-deficient mutant *aba1-3* and *abi1-1*, whereas a strong reduction in *AtPHO1;H10* expression occurred in *aba1-3* and *abi1-1* following dehydration. Together, these results reveal that OPDA can modulate gene expression via COI1 in a manner distinct from JA, and independently from ABA. Furthermore, the implication of the ABA pathway in coregulating *AtPHO1;H10* expression is dependent on the abiotic stress applied, being weak under wounding but strong upon dehydration.

Jasmonates, which include jasmonic acid (JA) and its methyl ester (MeJA), act as plant growth regulators and signal molecules involved in numerous development processes and responses to the environment (Farmer et al., 2003; Devoto and Turner, 2005; Schilmiller and Howe, 2005; Wasternack, 2007; Browse and Howe, 2008). A prominent role of jasmonates has been described in the response of plants to wounding, pathogen attack as well as water stress and UV damage. JA is also essential in anther development (Feys et al., 1994; Stinzi and Browse, 2000; He et al., 2002).

JA biosynthesis occurs through the octadecanoic acid pathway and is initiated by the oxidation of α-linolenic acid to 13-hydroperoxylinolenic acid (Schaller et al., 2005). This hydroperoxide is then dehydrated by allene oxide synthase (AOS) and cyclized by allene oxide cyclase to form the cyclopentenone (9*S*,13*S*)-12-oxo-phytodienoic acid (OPDA). The enzyme OPDA reductase 3 (OPR3) then reduces the pentacyclic ring double bond to form 3-oxo-2(2′(*Z*)-pentenyl)-cyclopentane-1-octanoic acid (OPC:8). OPC:8 then goes through three cycles of β-oxidation in the peroxisome to yield 3*R*,7*S*-JA, which can be converted to biologically active derivatives, such as jasmonyl-L-Ile (Staswick and Tiryaki, 2004).

Several components of the JA-mediated signal transduction cascade have been characterized. One key component is CORONATINE INSENSITIVE1 (COI1). COI1 contains an F-box domain and associates with Skp-like proteins, cullin, and AtRbx1, a RING-box 1 protein to form an active SCF^{COI1} complex that is thought to function as an E3-type ubiquitin ligase (Xu et al., 2001; Devoto et al., 2002). COI1 is required for numerous JA-dependent responses, including resistance to several pathogens and insects, and anther development (Feys et al., 1994; Xie et al., 1998). Recently, the JASMONATE ZIM (JAZ) domain transcriptional repressors have been identified as targets of the SCF^{COI1} complex and jasmonate treatment induces their degradation by proteasomes (Chini et al., 2007; Thines et al., 2007). Members of the JAZ protein family interact and negatively regulate MYC2, a key transcriptional activator of jasmonate responses (Chini et al., 2007). Mutations and natural truncations in JAZ family members lead to reduced sensitivity to JA and impairs resistance to insect herbivory (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007; Chung et al., 2008).

Apart from being a precursor to JA, OPDA has been found to be an active signal molecule. Transient increase of both OPDA and JA has been described in

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response to wounding and osmotic stress (Kramell et al., 2000; Reymond et al., 2000; Stinzi et al., 2001). Treatment of barley (*Hordeum vulgare*) leaves with OPDA led to the activation of genes that were not activated by endogenous JA (Kramell et al., 2000). Furthermore, tendril coiling of *Bryonia dioica* was found to be more responsive to OPDA then JA (Stelmach et al., 1998; Blechert et al., 1999). The Arabidopsis (*Arabidopsis thaliana*) *opr3* mutant accumulates OPDA following wounding but is deficient in JA biosynthesis (Stinzi et al., 2001). This mutant exhibits delayed anther dehiscence, resulting in male sterility (Sanders et al., 2000; Stinzi and Browse, 2000). Application of JA but not of OPDA can restore fertility, indicating that JA and not OPDA is the active molecule that regulates anther development. However, *opr3* plants were shown to be as resistant as wild type to both the dipteran *Bradysia impatiens* and the fungal pathogen *Alternaria brassicicola* (Stinzi et al., 2001). Through the analysis of a set of 150 defense-related genes, OPDA was found to not only up-regulate COI1-dependent genes that are also regulated by JA, but also induced several genes in a COI1-independent fashion that are not induced by JA (Stinzi et al., 2001). More recently, from an oligonucleotide array containing 21,500 Arabidopsis genes, a set of approximately 150 genes were identified that were induced by exogenous OPDA but not by exogenous JA or MeJA (Taki et al., 2005). Approximately half of these OPDA-specific response genes were induced by wounding, and analysis of a subset of six revealed that all were induced by OPDA in a COI1-independent fashion. Thus, no gene induced by OPDA in a COI1-dependant pathway, and not by JA, has been identified from these studies.

Numerous responses of plants that are mediated by jasmonates, such as wounding or defense against pathogens, are also influenced by other hormones that may interact in an antagonistic or synergistic fashion depending on the stress or developmental process (Lorenzo and Solano, 2005). Significant synergistic interactions between the ethylene and jasmonate pathways have been described for wounding, with the ETHYLENE RESPONSE FACTOR1 (ERF1) being a convergence point between these pathways (Lorenzo et al., 2003). The salicylic acid (SA)-mediated pathway can be either synergistic or antagonist to the JA pathway, depending on the concentration of SA and JA (Lorenzo and Solano, 2005; Mur et al., 2006). The abscisic acid (ABA) signal transduction pathway has been shown to contribute to the induction of *PIN2* (proteinase inhibitor II) and of other genes in tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum*) leaves following wounding, as well as to affect the feeding of insects on Arabidopsis (Pena-Cortes et al., 1995; Herde et al., 1996; Carrera and Prat, 1998; Bodenhausen and Reymond, 2007). However, the activation of several genes by JA were shown to be independent of ABA in barley and potato (Lee et al., 1996; Dammann et al., 1997). ABA interacts both in an antagonistic and synergistic fashion with JA in the response to distinct pathogens (Anderson et al., 2004; Mauch-Mani and Mauch, 2005; Adie et al., 2007).

This study describes the response of one member of the *PHO1* gene family in Arabidopsis to biotic and abiotic stresses. PHO1 is involved in the transfer of phosphate to the xylem in the root, and the Arabidopsis genome contains 10 additional genes showing homology to *PHO1* (named *AtPHO1;H1* to *AtPHO;H10*; Poirier et al., 1991; Hamburger et al., 2002; Wang et al., 2004). The gene *AtPHO1;H10* was found to be strongly induced by numerous stresses, including in the local response to wounding. The signal molecules and transduction pathways involved in the regulation of *AtPHO1;H10* following wounding and dehydration was thus investigated. *AtPHO1;H10* expression was induced by OPDA but not by JA or by coronatine, a bacterial polyketide metabolite that mimics jasmonates. Surprisingly, induction of *AtPHO1;H10* by both external OPDA application and wounding occurred via a COI1-dependent pathway. *AtPHO1;H10* was also induced by ABA in a COI1-independent manner, and both the ABA- and OPDA-mediated signal transduction pathways were involved in the induction of *AtPHO1;H10* following dehydration, while OPDA was the main signal involved in wounding.

RESULTS

AtPHO1;H10 Is Regulated by Numerous Biotic and Abiotic Stresses

Analysis by RNA gel-blot analysis of the expression of the *AtPHO1;H10* gene in soil-grown plants revealed that the gene was well expressed in roots and flowers but weakly in leaves and the inflorescence stems (Fig. 1A). A similar stronger expression in roots compared to leaves was observed for plants grown in agar-solidified medium (Fig. 1A). In transgenic plants expressing the GUS reporter gene under the control of 1.0 kb of the *AtPHO1;H10* promoter sequence (pH10::GUS line), strong GUS expression was evident in the epidermal and cortical cells of the roots, particularly in the primary roots and more weakly in the emerging secondary roots (Fig. 1, B and C). In the leaves, expression was primarily limited to the hydathodes and more weakly to trichomes (Fig. 1D).

Following an initial observation of strong GUS expression at the cutting edge of a petiole in pH10::GUS plants (data not shown), expression of *AtPHO1;H10* in wild-type plants treated with a variety of abiotic and biotic stresses was examined by RNA gel-blot analysis. In soil-grown plants, a strong accumulation of *AtPHO1;H10* mRNA was observed in leaves 2 h after mechanical wounding, 2 h after initiation of dehydration, 12 h after cold treatment at 4°C, 4 h after paraquat treatment, 18 h after infection of leaves with *Pseudomonas syringae* pv. *tomato* DC3000 expressing or not the avirulence gene avrRpm1, and in senescing leaves (Fig. 1E). Similarly, a strong expression of *AtPHO1;H10* in plants grown in agar-solidified medium was observed after 2 h of transfer to medium containing

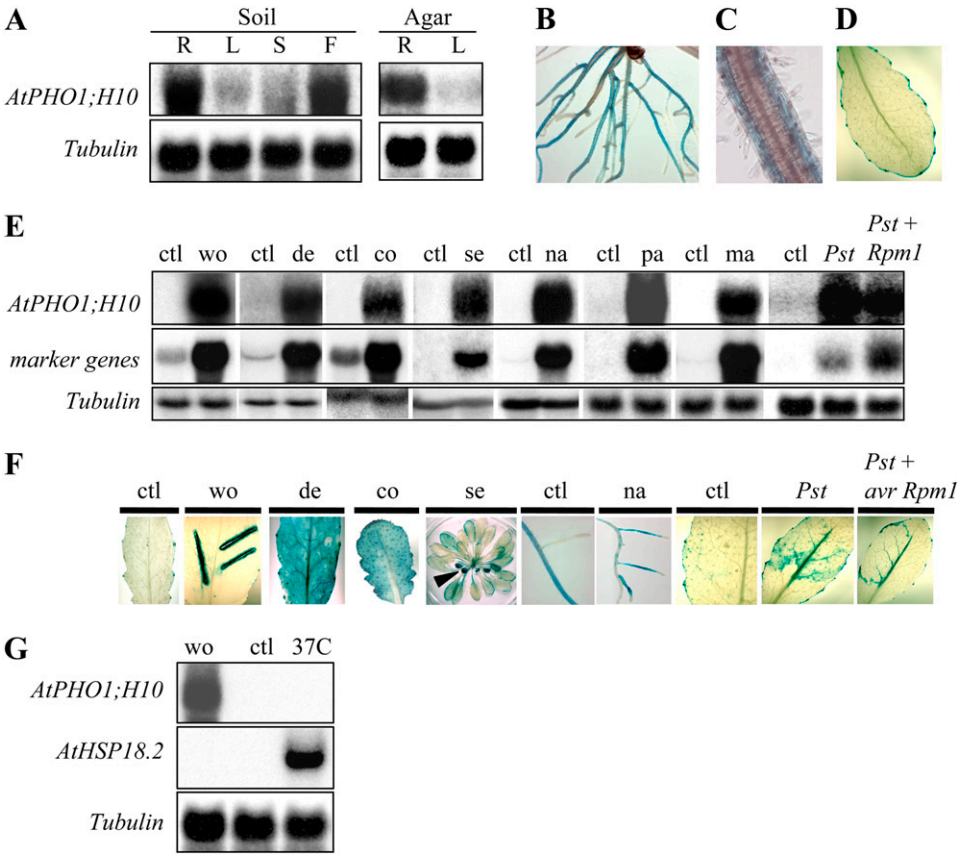

Figure 1. Regulation of *AtPHO1;H10* expression by various biotic and abiotic stresses. A, Expression of *AtPHO;H10* in roots (R), leaves (L), stems (S), and flowers (F) from soil-grown plants, or from leaves (L) and roots (R) from plants grown on agar. B to D, Pattern of expression of the GUS reporter gene in roots (B and C) and leaves (D) of ppH10::GUS plants. E, Comparison of the *AtPHO1;H10* mRNA levels between control untreated plants (ctl) and leaves 2 h after wounding with razor blade (wo), leaves 2 h after dehydration (de), leaves 12 h after a cold treatment at 4°C (co), senescent leaves (se), seedlings 2 h after transfer to medium containing100 mM NaCl (na), leaves 4 h after spraying of 10 μM paraquat (pa), seedlings 4 h after transfer to medium containing 250 mM mannitol (ma), and leaves 18 h after infection with *P. syringae* pv. *tomato* DC3000 (*Pst*) or *Pst* with the avirulence gene *avrRpm1*. All stresses were performed on the leaves of 6-week-old plants grown in soil at 20°C under short-day photoperiod (10-h day/14-h night), except for the NaCl and mannitol treatments, which were realized on the whole 10-d-old plants grown under continuous light at 20°C in agar-solidified medium containing half-strength MS and 1% Suc. Probes for marker genes induced by the various stresses were used as control. The *AtRD29A* gene was chosen to confirm stress induced by dehydration, cold, NaCl, and mannitol treatments. Similarly, *AtJR3* was used for the wounding stress, *AtSAG12* for senescence, *AtGST6* for the paraquat treatment, and *AtPR1* for the infection with *P. syringae*. F, Transgenic ppH10::GUS plants were treated by wounding (wo), dehydration (de), cold (co), addition of NaCl (na), infection by *P. syringae* pv. *tomato* DC3000 (*Pst*) or *Pst* with the avirulence gene *avrRpm1*, as indicated above. Senescing (se) cotyledons and first leaves from a 6-week-old rosette are indicated by the arrow. G, *AtPHO1;H10* mRNA levels in leaves after a heat stress at 37°C for 1 h. Probe for the heat shock gene *AtHSP18.2* was used as control for heat stress and RNA extracted from wounded leaves (wo) was used as a positive control for hybridization with the *AtPHO;H10* probe. For all RNA gel blots, 25 μg of total RNA was used and hybridization with an Arabidopsis α-tubulin probe was used as loading control.

100 mM NaCl or 4 h after transfer to 250 mM mannitol (Fig. 1E). Treatment of transgenic pH10::GUS plants with a subset of the stresses described above also showed strong GUS expression in either the roots (NaCl stress) or leaves (wounding, dehydration, senescence, and *P. syringae* infection [Fig. 1F]). However, no induction of *AtPHO1;H10* expression could be detected by RNA gel-blot analysis in leaves following a heat shock at 37°C for 1 h (Fig. 1G).

In pH10::GUS transgenic plants, GUS expression was restricted to the wound site for either leaves wounded mechanically with a razor blade, or challenged with the chewing caterpillar *Pieris rapae* (Fig. 2, B and D). RNA gel-blot analysis confirmed that the up-regulation of *AtPHO1;H10* expression in wounded or eaten leaves was restricted to the zone surrounding the wound site and not at a distal region in the same wounded leaves (Fig. 2, A and C).

AtPHO1;H10 Is Induced by ABA and OPDA But Not by JA or Coronatine

The induction of *AtPHO1;H10* following treatment of plants with various hormones and signal molecules

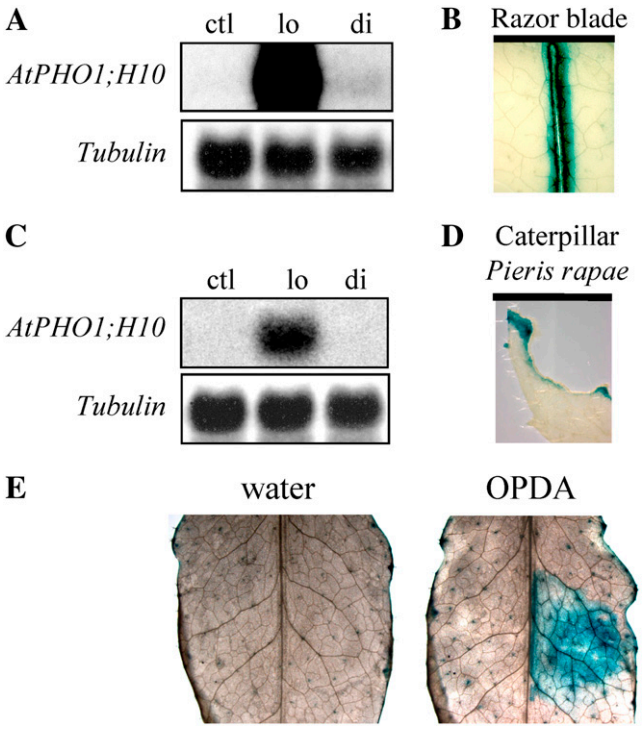

Figure 2. Local accumulation of *AtPHO1;H10* mRNA in leaves near the wound site and in areas infiltrated with OPDA. Wounding was performed on leaves either with a razor blade (A and B) or by feeding with the caterpillar *P. rapae* (C and D). RNA gel-blot anaylsis experiments (A and C) with RNA extracted from unwounded leaves (ctl), from tissues 3 mm around the wound site (lo, local), and from the remaining part of the wounded leaves (di, distal). B and D, Localization of GUS activity in leaves of transgenic ppH10::GUS plants following wounding with a razor blade (B) or by *P. rapae* feeding (D). Samples for northern or GUS staining were harvested 3 and 5 h after wounding by a razor blade or *P. rapae* feeding, respectively. E, The right side of the abaxial side of ppH10::GUS plants was infiltrated with either water (left) or 3 nmol of OPDA (right) and stained for GUS activity after 3 h.

involved in the response of plants to different biotic and abiotic stresses was examined by RNA gel-blot analysis. Treatment of plants with 100 μM ABA led to strong expression of *AtPHO1;H10*, with increased expression being detectable as early as 30 min after ABA addition and maximal expression observed at 2 h (Fig. 3A). Strong expression of *AtPHO1;H10* was also observed after 2 h of treatment with ABA concentrations ranging from 5 to 100 μM (Fig. 3B). Analysis of leaves 2, 4, and 8 h after infiltration with 1, 3, and 10 nmol of either JA or coronatine did not lead to the induction of *AtPHO1;H10*, whereas induction of the marker gene *AtVSP2* was observed for JA and coronatine, respectively (Fig. 3, C and D). In contrast, infiltration of leaves with OPDA led to overexpression of *AtPHO1;H10*, with maximal expression observed after 3 h of infiltration with 3 nmol of OPDA (Fig. 3E). Infiltration of leaves of pH10::GUS plants with 3 nmol OPDA led to a local induction of GUS in the infiltrated area (Fig. 2E). No induction of *AtPHO1;H10* expression was observed after 3 h of infiltration of leaves with 10 nmol oleic acid or linolenic acid (data not shown). Similarly, no induction of *AtPHO1;H10* expression was observed in plants treated with methyl salicylate (Fig. 3F).

Induction of *AtPHO1;H10* by Wounding Is Mediated by OPDA via a COI1-Dependent Pathway

Induction of *AtPHO1;H10* by wounding was analyzed in various mutants affected in the jasmonate biosynthetic or perception pathway. The *aos* mutant is defective in the *AOS* gene, and is deficient in both OPDA and JA synthesis (Park et al., 2002). In contrast, the *opr3* mutant is defective in the gene encoding the peroxisomal OPDA reductase 3 and is able to accumulate OPDA but not JA (Stinzi and Browse, 2000; Stinzi et al., 2001). Finally, the *coi1-1* mutant is deficient in numerous JA-mediated responses (Feys et al., 1994; Xie et al., 1998).

Overexpression of *AtPHO1;H10* following mechanical wounding was nearly abolished in both the *aos* and *coi1-1* mutant while it remained unaffected in the *opr3* mutant (Fig. 4A), indicating that endogenous OPDA synthesized following wounding was capable of inducing *AtPHO1;H10* expression via a COI1-dependent pathway. Furthermore, induction of *AtPHO1;H10* expression by infiltration of OPDA was also abolished in the *coi1-1* mutant (Fig. 4B).

Wounding involves dehydration at the wound site and may involve an ABA-mediated response (Reymond et al., 2000). Induction of *AtPHO;H10* following mechanical wounding was thus analyzed in the *aba1-3* mutant deficient in ABA synthesis (Koornneef et al., 1982), and in the ABA-insensitive mutant *abi1-1*, deficient in multiple responses of plants to ABA (Koornneef et al., 1984). For both *aba1-3* and *abi1-1* mutants, the overexpression of *AtPHO1;H10* following wounding was unchanged compared to the control (Fig. 4C).

AtPHO1;H10 Expression under Dehydration Involves Both ABA and OPDA-Mediated Signaling

The contribution of both the jasmonate- and ABA-mediated signaling pathway to the induction of *AtPHO1;H10* expression following dehydration of rosette was assessed. Induction of *AtPHO1;H10* was strongly reduced in both the *aos* and *coi1-1* mutants, whereas only a slight reduction was observed in the *opr3* mutant (Fig. 5A). A strong reduction in *AtPHO1;H10* expression following dehydration was also observed in the *aba1-3* and *abi1-1* mutant (Fig. 5B). Together, these results indicate a contribution of both OPDA and ABA to the response of *AtPHO1;H10* to dehydration.

AtPHO1;H10 Expression by ABA Involves the ABI1 and ROP10 Signaling Pathway and Is Independent of the Jasmonate Pathway

Induction of *AtPHO1;H10* expression by ABA in seedlings was strongly suppressed in *abi1-1* (Fig. 6A).

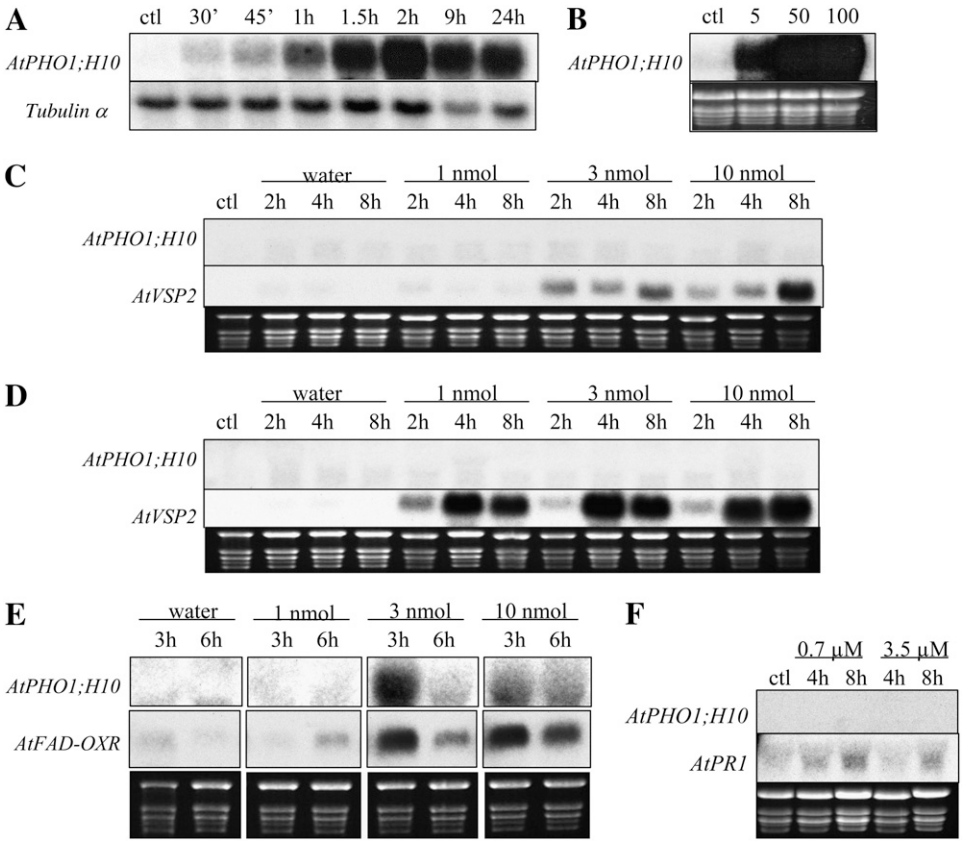

Figure 3. Regulation of *AtPHO1;H10* transcript levels by exogenous addition of phytohormones. A, Plants grown on agar-solidified medium for 7 d were transferred to the same medium containing 100 μM ABA for times ranging from 30 min up to 24 h before being harvested. Control (ctl) plants were transferred to agar-solidified medium without ABA for 24 h. B, Plants grown on agar-solidified medium for 7 d were transferred to the same medium containing no ABA (ctl) or 5, 50, or 100 μM ABA for 2 h before being harvested. Leaves of plants grown in soil were infiltrated with either 10 μL of water or water containing 5% ethanol, or 10 μL of solutions containing JA (C), coronatine (D), or OPDA (E), representing the infiltration of 1, 3, or 10 nmol of jasmonates. Infiltrated leaves were harvested between 2 and 8 h postinfiltration. Control leaves (ctl) were not infiltrated. F, Plants were placed in boxes containing the volatile methyl salicylate at a final concentration in air of 0.7 or 3.5 μM and leaves harvested after 4 to 8 h. Control plants (ctl) were put in boxes without methyl salicylate for 8 h. Probes used for hybridization are indicated on the left of every panel.

ROP10 is a member of the ROP subfamily of Rho GTPases located in the plasma membrane and was shown to negatively regulate ABA-mediated responses (Zheng et al., 2002). The *rop10* mutant was previously shown to exhibit enhanced responses to ABA and the induction of *AtPHO1;H10* by ABA was enhanced in the *rop10* mutant compared to wild-type plants (Fig. 6B; Zheng et al., 2002).

Analysis of 1 kb of promoter sequence of *AtPHO1;H10* revealed the sequences CACGTGTC and CACGTGGC 619 and 508 bp upstream of the start codon, respectively, which conforms to the cis-acting ABA responsive elements (ABREs). Furthermore, the sequence CACGCGT, which conforms to the CE3 coupling element, is found 23 bp upstream of the CACGTGGC ABRE (Rock, 2000). These sequences are recognized by members of the bZIP (basic-domain Leu zipper) transcription factors, which include ABF3, ABF4, and ABI5, three proteins involved in ABA-mediated responses (Rock, 2000; Yamaguchi-Shinozaki and Shinozaki, 2004). Expression of *AtPHO1;H10* was analyzed in transgenic lines overexpressing the ABF3, ABF4, and ABI5 transcription factors under the control of the CaMV35S promoter (Kang et al., 2002; Lopez-Molina et al., 2002). In plants treated or untreated with ABA, overexpression of *AtPHO1;H10* relative to wild-type plants was only observed in the ABI5-overexpressing lines (Fig. 6C). For the ABF3-overexpressing line, *AtPHO1;H10* expression following ABA treatment was reduced compared to control (Fig. 6C). Finally, the overexpression of *AtPHO1;H10* following ABA treatment was found to be unaffected in the *aos* and *coi1-1* mutant (Fig. 6D).

DISCUSSION

The observed pattern of induction of *AtPHO1;H10* expression by multiple stresses is largely in agreement with its induction by either exogenous ABA or OPDA

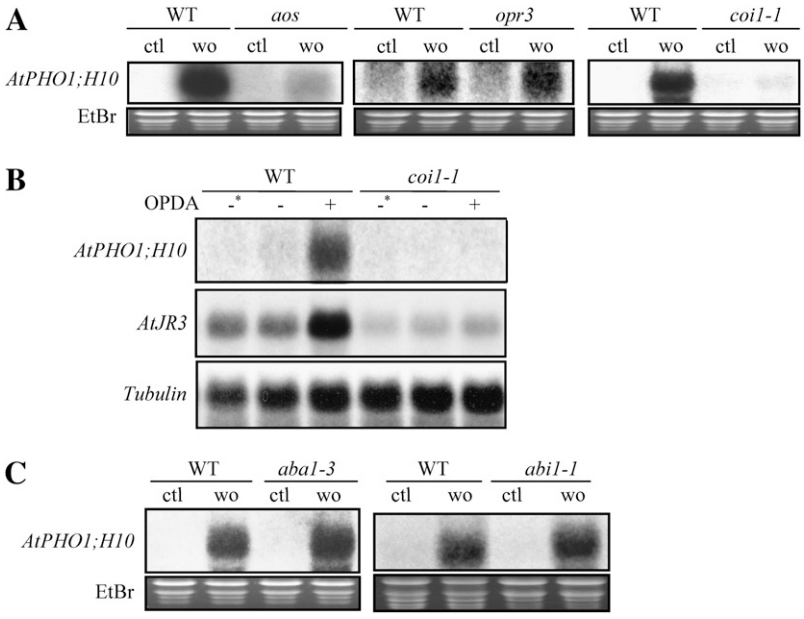

Figure 4. Regulation of the *AtPHO1;H10* expression following wounding and OPDA treatment in JA and ABA mutants. A and C, RNA was extracted from unwounded leaves (ctl) or leaves 2 h after wounding with a razor blade (wo). B, RNA was extracted from uninfiltrated leaves (−*), leaves 3 h following infiltration with water (−), or 3 h following infiltration with 10 nmol OPDA (+). Wild-type plants used as control were of the same accession genotype as the corresponding mutants, namely Col for the *aos* and *coi1-1* mutants, Ws for *opr3,* and L*er* for *aba1-3* and *abi1-1*.

applications. Changes in the endogenous level of ABA and the implication of the ABA signaling pathway has been well described for stresses mediated by wounding, dehydration, cold, senescence, salt, and osmotic stress (Pena-Cortes et al., 1989, 1995; Birkenmeier and Ryan, 1998; Zhu, 2002; Gusta et al., 2005; de Torres-Zabala et al., 2007). ABA has also been implicated in the response of plants to pathogen infection, including infection with *P. syringae* (Anderson et al., 2004; Mauch-Mani and Mauch, 2005; Adie et al., 2007; de Torres-Zabala et al., 2007). Similarly, increase in endogenous level of OPDA and/or JA, as well as the implication of the JA signaling pathway has been described for wounding, dehydration, senescence, salt, osmotic, and oxidative stresses, as well as for infection with pathogens (Harms et al., 1995; Lehmann et al., 1995; Pena-Cortes et al., 1995; Herde et al., 1996; Moons et al., 1997; Reymond et al., 2000; Stinzi and Browse, 2000; He et al., 2002; Sasaki-Sekimoto et al., 2005; Truman et al., 2007). Thus, although it is not excluded that other molecules or signaling pathways could be involved in modulating the expression of *AtPHO1;H10* in other stresses, these results reveal that both ABA and OPDA are important regulators for this gene.

Previous studies have shown that OPDA can have a biological activity that can either overlap with JA or be distinct from JA. Overlap in the activity of OPDA and JA has been shown by the maintenance of resistance to the dipteran *B. impatiens* and fungus *A. brassicicola* in the Arabidopsis mutant *opr3*, synthesizing OPDA but not JA (Stinzi et al., 2001). In contrast, the defect in pollen maturation observed in the *opr3* mutant can be complemented only by exogenous application of JA, indicating that JA and not OPDA is the active molecule that regulates anther development (Stinzi and Browse, 2000). Similarly, the ozone-treated *opr3* mutant failed to activate several JA-regulated genes that are involved in antioxidant metabolism, and the *opr3* mutant was more sensitive to ozone exposure compared to wild type (Sasaki-Sekimoto et al., 2005). At the genetic level, the combination of gene expression studies between the wild type and *opr3* mutants following wounding or infiltration with either OPDA or JA has revealed genes, such as *JR3* (At1g51760) and *HPL* (At4g15440), which can be activated either by

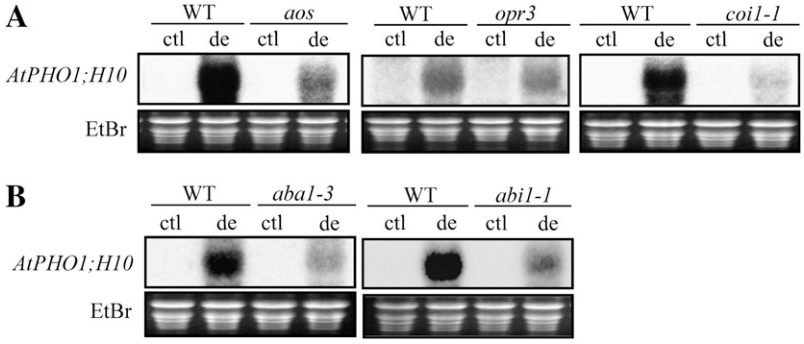

Figure 5. Regulation of the *AtPHO1;H10* expression following dehydration in JA and ABA mutants. RNA was extracted from leaves of well-watered plants (ctl) or leaves 2 h after dehydration was induced by cutting a rosette at the hypocotyl and placing it on a filter paper. Wild-type plants used as control were of the same accession genotype as the corresponding mutants, namely Col for the *aos* and *coi1-1* mutants, Ws for *opr3,* and L*er* for *aba1-3* and *abi1-1*.

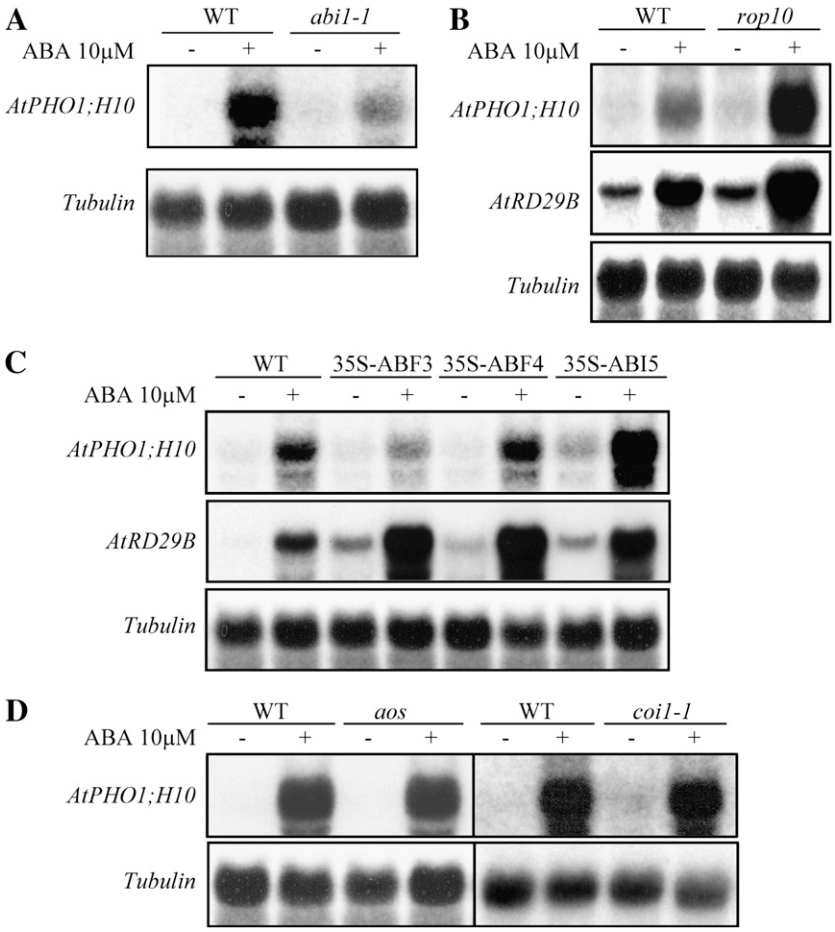

Figure 6. Regulation of the *AtPHO1;H10* expression by exogenous treatment with ABA in mutants or transgenic plants affected in JA or ABA responses. Plants were grown for 10 d in agar-solidified medium containing half-strength MS and 1% Suc before being transferred to the same medium supplemented with 10 μM ABA for 2 h. RNA was extracted from the whole plants. Wild-type plants used as control were of the same accession genotype as the corresponding mutants, namely L*er* for the *abi1-1* mutant (A) and the transgenic lines 35S-ABF3, 35S-ABF4, and 35S-ABI5 (C), Ws for *rop10* (B), and Col for the *aos* and *coi1-1* mutants (D). Probes used for hybridization are indicated on the leaf of every panel.

OPDA or JA, both in a COI1-dependent manner (Stinzi et al., 2001; Taki et al., 2005). However, some genes, such as *VSP2* (At5g24770) and *MBP* (At3g16460), were activated by JA and not OPDA, also in a COI1-dependent manner (Stinzi et al., 2001; Taki et al., 2005). Approximately 150 OPDA-regulated genes (ORGs) in Arabidopsis were found to be activated by exogenous OPDA but not JA (Stinzi et al., 2001; Taki et al., 2005). Expression analysis of a subset of ORGs, including *ERF5* (At5g47230) and *FAD-OXR* (At4g20860), revealed that all were activated via a COI1-independent pathway, clearly indicating that ORGs are activated via a mechanism distinct from the JA-COI1 pathway (Stinzi et al., 2001; Taki et al., 2005). In contrast, this work reveals that *AtPHO1;H10* can be activated by OPDA in a COI1-dependent manner, but not by JA. Together, these studies reveal that the output of the COI1-dependent pathway on gene activation can be quite distinct depending on whether OPDA or JA is involved (Fig. 7).

In accordance with the participation of JAZ proteins in the jasmonate signaling network, altered expression of some JAZ proteins lead to reduced sensitivity to JA (Chini et al., 2007; Thines et al., 2007; Yan et al., 2007). Physical interaction between COI1 and JAZ3/JAI3 or JAZ1 has been demonstrated in cell-free protein extracts, while interaction between JAZ1 and COI1 was further demonstrated in a yeast two-hybrid system (Chini et al., 2007; Thines et al., 2007). Remarkably, interaction between JAZ1 and COI1 was found to be dependent on the presence of JA-Ile, whereas OPDA, JA, or MeJA did not promote interactions between these proteins (Thines et al., 2007). Furthermore, proteasome degradation of JAZ3/JAI3 was impaired in the *jar1* mutant deficient in the enzyme responsible for the synthesis of JA-Ile conjugate (Chini et al., 2007).

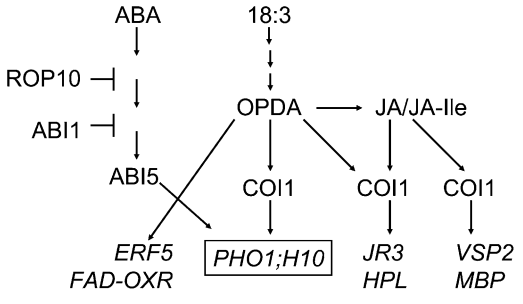

Figure 7. Model showing the distinct regulation of gene expression mediated by OPDA and JA via COI1-dependent and -independent pathways. A selection of genes activated by each pathway is indicated and is based on this work and the studies of Taki et al. (2005) and Stinzi et al. (2001).

Together, these results reveal that JA-Ile is an active hormone promoting the interaction between COI1 and either JAZ1 or JAZ3/JAI3. Furthermore, although OPDA was previously regarded as a close structural analog of coronatine (Weiler et al., 1994), the current data rather suggest that coronatine is a structural analog of JA-Ile (Staswick, 2008). In this context, what then is the significance of the activation of *AtPHO1;H10* by OPDA and not JA or coronatine, in a COI1-dependent fashion? One hypothesis would be that OPDA promotes the interaction of COI1 with a distinct member of the JAZ protein family, which comprises 12 members in Arabidopsis. Alternatively, OPDA may activate *AtPHO1;H10* through the interaction of COI1 with other unidentified proteins. The sets of genes regulated by COI1 would thus depend on both the nature of the jasmonate (e.g. OPDA or JA-Ile) and the presence of distinct effector molecules interacting with COI1. The distinct role of jasmonates other than JA-Ile has recently been supported by findings that wound-induced expression of COI1-dependent genes in the Arabidopsis JA-Ile-deficient *jar1-1* mutant is not significantly impaired, and by the distinct role of JA and JA-Ile in herbivore resistance of *Nicotiana attenuata* (Wang et al., 2007; Chung et al., 2008).

Several physiological and developmental processes are influenced by either a synergistic or antagonistic cross talk between ABA and jasmonates, including seed germination (Wilen et al., 1991; Staswick et al., 1992; Ellis and Turner, 2002) and defense against pathogens (Anderson et al., 2004; Mauch-Mani and Mauch, 2005; Adie et al., 2007). Positive interactions between ABA and JA have been reported for wounding, mainly in potato and tomato. ABA synthesis or perception via ABI1 was required for the local increase in JA and the induction of *PIN2* expression following wounding in both potato and tomato (Pena-Cortes et al., 1989, 1995, 1996; Hildmann et al., 1992; Herde et al., 1996; Carrera and Prat, 1998). Increases in ABA were also measured following wounding in both local and systemic leaves, and the expression of several JA-regulated genes, including *PIN2*, in the wounded ABA-deficient mutant can be restored either via ABA or JA application (Pena-Cortes et al., 1989, 1995; Creelman and Mullet, 1995; Herde et al., 1996; Birkenmeier and Ryan, 1998). Together, these studies indicated that in potato and tomato, ABA synthesis and perception was necessary for activation of the JA biosynthetic and signaling pathway following wounding. In Arabidopsis, ABA was found to activate *AtMYC2* in a COI1-dependent manner, indicating that ABA precedes JA in the activation of *AtMYC2*-mediated wound responses (Lorenzo et al., 2004). It was, however, noted that distinct genes can be induced by either ABA or JA in various tissues in potato and barley (Lee et al., 1996; Dammann et al., 1997), and that a higher level of ABA can be maintained in dehydrated tissues without activation of JA synthesis or JA-regulated genes in soybean (*Glycine max*) and tomato (Creelman and Mullet, 1995; Birkenmeier and Ryan, 1998).

Activation of *AtPHO1;H10* expression by external ABA was independent of the presence of both AOS and COI1, but was strongly influenced by the presence of ABI1 and ROP10, indicating the lack of involvement of the jasmonate pathway and the main participation of the ABA signaling pathway in this activation. The presence of ABRE and C3 cis-acting elements in the promoter of *AtPHO1;H10* and the activation of *AtPHO1;H10* expression in the transgenic line overexpressing the ABI5 transcription factor further highlight the main contribution of the ABA signaling pathway via ABI5 in *AtPHO1;H10* regulation. The lack of response of the *AtPHO1;H10* gene to overexpression of ABF3 and ABF4 is in contrast to the strong activation of *AtPHO1;H10* by ABI5 (Fig. 6B). These data likely reflect differential strength of interaction of these three transcription factors to the cis-promoter elements present in *AtPHO1;H10*.

The contribution of the ABA signaling pathway to *AtPHO1;H10* expression following wounding and dehydration was distinct. Thus, *AtPHO1;H10* induction by wounding was found to be unaffected in the *aba1-3* and *abi1-1* mutants, indicating that in contrast to the ABA-dependent activation of *PIN2* in wounded potato and tomato leaves, induction of *ATPHO1;H10* expression by wounding mainly involved the OPDA-COI1 pathway and was independent of the ABA pathway. In contrast, upon dehydration, both the ABA and OPDA-COI1 pathway needed to be present to achieve maximal *AtPHO1;H10* expression. The implication of ABA upon dehydration could perhaps be linked to a higher increase in ABA level in dehydrated tissues compared to wounding (Creelman and Mullet, 1995; Birkenmeier and Ryan, 1998). Interestingly, a role for ABA in defense against insect herbivory has been demonstrated, with the weight of *Spodoptera littoralis* larvae feeding on the ABA-deficient mutant *aba2-1* being higher than larvae feeding on wild-type plants (Bodenhausen and Reymond, 2007). The same study revealed that a number of ABA-regulated genes are induced upon insect feeding either in a COI1-dependent or COI1-independent manner. Together, these results highlight that the extent of the interaction between the ABA and jasmonate pathways in biotic and abiotic stress is highly dependent on the nature of the stress involved. Similar conclusions are also emerging from the study of ABA and jasmonate signaling pathways in plant-pathogen interactions, whereby ABA can be either play a synergistic or antagonistic role with jasmonates depending on the pathogen (Anderson et al., 2004; Mauch-Mani and Mauch, 2005; Adie et al., 2007).

At present, the role of *AtPHO1;H10* in the plant's response to wounding or water stress remains unclear. Although both *AtPHO1* and its closest homolog *AtPHO1;H1* are involved in the loading of inorganic phosphate to the xylem (Stefanovic et al., 2007), the distinct pattern of expression of *AtPHO1;H10* makes it unlikely that it could have a similar role in Pi export. Interestingly, the *ATPHO1;H4* gene has been implicated

in the response of the hypocotyl to blue light, indicating that AtPHO1 family members could be involved in signal transduction cascades associated with different environmental stimuli (Ni and Kang, 2006). However, comparison of gene expression using complementary DNA (cDNA) microarray between the wild type and T-DNA null mutant for *AtPHO1;H10* in response to abiotic wounding (3 h after wounding) or exogenous ABA treatment (1 and 12 h after addition of 100 μM ABA) has failed to reveal differences that could implicate *AtPHO1;H10* in early events in ABA or OPDA signal transduction cascade (data not shown). Nevertheless, the strong and localized expression of *AtPHO1;H10* at the wound site provides a novel reporter for the study of cell signaling in the immediate vicinity of the wound. This zone, restricted to only a few cells, is particularly susceptible to dehydration and has a direct contact to the atmosphere, distinguishing it from leaf zones distal to the wound. The unique regulation of *AtPHO1;H10* provides a valuable marker to study the factors that enable distinct signaling cascades to emerge from the SCF^{COI1} complex depending on the presence of either OPDA or JA.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Seeds of wild-type Arabidopsis (*Arabidopsis thaliana*) accession Columbia (Col), Landsberg *erecta* (L*er*), or Wassilewskija (Ws), as well as of mutants (ecotypes of the mutants are indicated in parenthesis) *aos* (Col; Park et al., 2002), *opr3* (Ws; Stinzi and Browse, 2000), *coi1-1* (Col; Feys et al., 1994), *aba1-3* (L*er*; Koornneef et al., 1982), *abi1-1* (L*er*; Koornneef et al., 1984), 35S-ABF3 (L*er*; Kang et al., 2002), 35S-ABF4 (L*er*; Kang et al., 2002), 35S-ABI5 (L*er*; Lopez-Molina et al., 2002), and *rop10* (Ws; Zheng et al., 2002) were sown in 7-cm-diameter pots containing potting compost and vernalized for 4 d at 4°C. Plants were then grown for 6 to 7 weeks in a growth room (20°C at 70% relative humidity and with 10-h/14-h light/dark cycle at 100 $\mu Em^{-2} s^{-1}$). For some experiments, plants were grown in agar-solidified half-strength Murashige and Skoog (MS) medium under constant illumination.

Wounding, Dehydration, Pathogen, and Chemical Treatments

Wounding was done with incisions made with a razor blade across the whole surface at intervals of approximately 2 mm. The main vein and the edges of the leaf were left undamaged. Following wounding, plants were kept in the same growth room for 3 h before being harvested and frozen in liquid nitrogen. For biotic wounding experiments, *Pieris rapae* caterpillars were placed on plants and were allowed to feed under light for 5 h at 20°C until approximately 40% of the leaf surface was removed. Larvae were then removed, and all plant leaves were immediately frozen in liquid nitrogen or used for GUS assay. Dehydration was performed by excising the whole rosettes from their roots with a razor blade and then placing them on a paper in the same growth chamber at 20°C and 70% humidity for various times before freezing in liquid nitrogen. Stock solutions of 10 mM (±)-cis, trans-ABA (Sigma) was prepared in water with a few microliters of NaOH 1 N to help the dissolution. Solutions of 9*S*,13*S*-OPDA (Larodan), coronatine (Sigma), and the (±)-JA (Sigma) were infiltrated into the abaxial surface of the leaf with a syringe without a needle, at 10 μL per leaf. Before infiltration of OPDA, the ethanol was evaporated and the residues were dissolved in a volume of ethanol adjusted so that the final concentration of ethanol after water addition was 5% (v/v). To improve solubilization, OPDA solution was sonicated in a water bath. JA and coronatine were directly dissolved into water. Control leaves were infiltrated with water or water containing the same concentration of ethanol. Volatile methyl-salicylate (Sigma) treatments were done in hermetic plexiglass boxes (11.4 L) by applying either 7.9 μmol or 40 μmol of MeJA on a Q-tip (final concentration of 0.7 μmol or 3.5 μmol to 1 L of air volume, respectively). For infection of leaves with *Pseudomonas syringae* pv. *tomato* DC3000 with or without the avirulence gene *avrRpm1*, the abaxial surface of leaves was infiltrated with 2.5×10^5 colony-forming units in 10 μL of 10 mM $MgCl_2$ using a syringe without a needle.

RNA Isolation and Northern Hybridization Analysis

Total RNA was extracted from plants tissues by phenol:chloroform separation and lithium chloride precipitation followed by washes with sodium acetate and ethanol as previously described (Reymond et al., 2000). Northern analysis was performed by separating 25 μg of total RNA on agarose gels containing formaldehyde, transferring to nylon membranes (Hybond N+; Amersham Biosciences), and hybridizing with P^{32}-radiolabeled specific probes according to standard procedures under high stringency conditions. Specific probes corresponded to the gene-specific tags (designed in the microarray CATMA project; Crowe et al., 2003) amplified by PCR with a specific set of primers, or to full-length cDNA.

GUS Staining

Transgenic plants expressing the *uidA* reporter gene under the control of a 1-kb fragment of the *AtPHO1;H10* promoter (Wang et al., 2004) were selected in agar-solidified medium containing 50 μg/mL of kanamycin. The resistant plants were then transferred in pots containing fertilized soil for 5 weeks. Rosettes were stained for GUS activity as previously described (Wang et al., 2004).

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