Running Head: IOS1 downregulates ABA responses in Arabidopsis

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The Receptor Kinase IMPAIRED OOMYCETE SUSCEPTIBILITY 1 Attenuates Abscisic Acid Responses in Arabidopsis thaliana

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Summary: The Arabidopsis receptor-like kinase IOS1 negatively regulates abscisic acid hormone signaling and promotes infection by filamentous (hemi-)biotrophs.
Footnotes:

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ABSTRACT

In plants, membrane-bound receptor kinases are essential for developmental processes, immune responses to pathogens and the establishment of symbiosis. We previously identified the Arabidopsis (Arabidopsis thaliana) receptor kinase IMPAIRED OOMYCETE SUSCEPTIBILITY 1 (IOS1) as required for successful infection with the downy mildew pathogen, Hyaloperonospora arabidopsidis. We report here that IOS1 is also required for full susceptibility of Arabidopsis to unrelated (hemi-)biotrophic filamentous oomycete and fungal pathogens. Impaired susceptibility in the absence of IOS1 appeared to be independent of plant defense mechanism. Instead, we found that ios1-1 plants were hypersensitive to the plant hormone abscisic acid (ABA), displaying enhanced ABA-mediated inhibition of seed germination, root elongation, and stomatal opening. These findings suggest that IOS1 negatively regulates ABA signaling in Arabidopsis. The expression of ABA-sensitive COR and RD genes was diminished in Arabidopsis during infection. This effect on ABA signaling was alleviated in the ios1-1 mutant background. Accordingly, ABA-insensitive and ABA-hypersensitive mutants were more susceptible and resistant to oomycete infection, respectively, showing that the intensity of ABA signaling affects the outcome of downy mildew disease. Taken together, our findings suggest that filamentous (hemi-)biotrophs attenuate ABA signaling in Arabidopsis during the infection process, and that IOS1 participates in this pathogen-mediated reprogramming of the host.
INTRODUCTION

Membrane-bound receptor-like kinases (RLKs) perceive molecules that mediate cell-to-cell communication during plant development, sense the biotic and abiotic environment and transduce the perceived stimuli into complex downstream signaling networks. Hormones govern most of the essential events during vegetative and reproductive plant growth, and some RLKs are hormone receptors, integrating stress stimuli into adapted defense responses. The best-known examples are BRASSINOSTEROID INSENSITIVE 1 (BRI1) and its co-RLK, BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1), which concertedly perceive brassinosteroid hormones. BAK1 also associates with pattern-recognition receptors (PRRs) that detect pathogens and induce innate immune responses (Chinchilla et al., 2009). brassinosteroids tune these responses through synergistic and antagonistic effects on the immune signaling network triggered by the bacterial patterns recognized (Albrecht et al., 2012; Belkhadir et al., 2012). Another example involves PHYTOSULFOKINE RECEPTOR 1 (PSKR1). Upon perception of the phytosulfokine hormone, PSKR1 modulates cellular de- and redifferentiation during organ development (Matsubayashi et al., 2002), but also integrates into the defense signaling network upon pathogen infection (Igarashi et al., 2012; Mosher et al., 2013). It was speculated that the principal function of PSKR1 signaling is to attenuate stress responses of cells during differentiation processes, such as vascularization (Motose et al., 2009). Concomitantly, PSKR1 affects the hormonal crosstalk that regulates plant-pathogen interactions. RLKs also perceive cytokinins (Inoue et al., 2001) and ethylene (Hua and Meyerowitz, 1998). Other hormones, however, such as auxins (Tan et al., 2007), gibberellins (Ueguchi-Tanaka et al., 2005) and abscisic acid (ABA) interact with intracellular rather than membrane-bound receptors. ABA recognition at the membrane has been discussed (Liu et al. 2007), but it is now well established that the hormone binds to PYRABACTIN RESISTANCE (PYR)/PYR1-LIKE (PYL)/REGULATORY COMPONENT OF ABSCISIC ACID RECEPTOR (RCAR), leading to the formation of stable complexes with type 2C protein phosphatases (PP2Cs) and to their subsequent inhibition (reviewed by Cutler et al., 2010). These complexes form the early ABA signaling module, which interacts with ion channels, transcription factors and other targets, providing a mechanistic connection between the phytohormone and ABA-induced responses. ABA responses are fine-tuned through a network of regulators including membrane receptors that sense developmental processes and environmental conditions. Examples include FERONIA (FER; Yu et al.,
2012), A4 lectin receptor kinases (Xin et al., 2009) and CYSTEINE-RICH RLK 36 (CRK36; Tanaka et al., 2012), all of which downregulate ABA signaling. Other sensors of environmental stress, such as PROLINE-RICH EXTENSIN-LIKE RECEPTOR KINASE 4 (PERK4; Bai et al., 2009) and RECEPTOR-LIKE PROTEIN KINASE 1 (RPK1; Osakabe et al., 2005), act in the opposite direction, upregulating ABA signaling. ABA signaling modulates pathogenesis in diverse plant-pathogen interactions and is involved in the crosstalk with plant defense pathways (Robert-Seilaniertz et al., 2011).

We previously identified the RLK IMPAIRED OOMYCETE SUSCEPTIBILITY 1 (IOS1, AT1G51800) as required for the full susceptibility of Arabidopsis thaliana to the downy mildew pathogen, Hyaloperonospora arabidopsidis (Hpa; Hok et al., 2011). IOS1 belongs to a subfamily of ~50 RLKs in Arabidopsis, which possess an extracellular region composed of a malectin-like domain (MLD) in addition to (2 to 3) leucine-rich repeats (LRRs; Hok et al., 2011). Malectin was first identified in Xenopus laevis as an ER-localized carbohydrate-binding protein (Schallus et al., 2008), which controls the N-glycosylation status of secreted glycoproteins (Chen et al., 2011). The role of MLDs in plants, notably as elements of the extracellular region of RLKs, is yet unknown (Lindner et al., 2012). The protein sequence and domain organisation of IOS1 have strong similarities with the SYMBIOSIS RECEPTOR-LIKE KINASE (SYMRK) and SYMRK-like RLKs from legumes, which are key regulators of the accommodation of fungal and bacterial symbionts (Stracke et al., 2002; Capoen et al., 2005; Markmann et al., 2008). Similar to SYMRK, IOS1 contains the conserved sequence GDPC within the interspace between MLD and LRRs of the extracellular domain (Hok et al., 2011). This motif is required for establishing the epidermal symbiotic program in roots, but not the subsequent cortical symbiosis, suggesting the perception of distinct signals by the extracellular domain of SYMRK during the compatible plant-microbe interaction (Kosuta et al., 2011). IOS1 was recently shown to form a complex with the pattern recognition receptors FLAGELLIN SENSING 2 (FLS2) and EF-TU RECEPTOR (EFR), as well as with BAK1, and to prime pattern-triggered immunity (PTI) to the bacterium Pseudomonas syringae (Chen et al., 2014). However, during the interaction of Arabidopsis with Hpa, IOS1 supports the success of downy mildew infection rather than PTI (Hok et al., 2011). The physiological programs, which are regulated by IOS1 in this interaction, are yet unknown.

Here, we investigated the function of IOS1 during the interaction with Hpa and unrelated filamentous biotrophic and hemibiotrophic pathogens. Collectively, our results
show that IOS1 downregulates ABA responses, thereby creating an environment that favors the biotrophic lifestyle of these pathogens.
RESULTS

IOS1 promotes infection by filamentous (hemi-)biotrophs

The transcription of IOS1 was activated by the infection of cotyledons with Hpa (Hok et al., 2011). This activation occurred locally at the early appressorium-mediated penetration stages of infection (Fig. 1A) in the single-cell layers surrounding the invading hyphae, which harbor haustoria (Fig. 1B). We further analyzed whether infection-responsive transcription of the IOS1 gene was restricted to leaf tissues, by inoculating Arabidopsis roots with the hemibiotrophic oomycete pathogen, Phytophthora parasitica (Pp). In non-inoculated roots of the IOS1 reporter line, IOS1 expression was only observed in the elongation zone, but not in the root tip (Fig. 1C). Upon inoculation, Pp zoospores attach to the root tip, penetrate the root via an appressorium, grow towards the central cylinder, and invade the roots and aerial organs (Attard et al., 2010). The transcription of IOS1 was activated during the initial biotrophic phase of Pp invasion (Fig. 1C) and the development of disease was significantly delayed in the ios1-1 loss-of-function mutant (Fig. 1D). The activation of IOS1 transcription by both leaf and root oomycete pathogens thus appears to be required for successful infection. A similar observation was made with the unrelated powdery mildew fungus, Erysiphe cruciferarum (Ec). On Arabidopsis leaves, germinating Ec conidia develop appressoria, which produce penetration pegs and haustoria within leaf epidermal cells, while the fungal mycelium grows on the leaf surface. During Ec infection, IOS1 transcription was activated in the mesophyll cells underlying the epidermal infection sites (Supplemental Fig. S1A). IOS1 expression in the mesophyll appeared to favor the progression of disease, as inoculated plants from the ios1-1 mutant line exhibited less powdery mildew symptoms on leaves than wild-type (Wt) plants (Supplemental Fig. S1B). Accordingly, significantly less conidiophores were produced on the ios1-1 mutant than on the Wt (Supplemental Fig. S1C). The observed interaction phenotypes with mildews are not caused by growth defects of the mutant, as vegetative development of ios1-1 was indistinguishable from the Wt (Supplemental Fig. S2). Wt and mutant plants were equally susceptible to root inoculations with the wilt bacterium, Ralstonia solanacearum (Supplemental Fig. S1D), indicating that the development of bacterial wilt occurs independently of IOS1. Taken together, our findings suggest that the host receptor IOS1 contributes to successful leaf- and root invasions by filamentous (hemi-)biotrophs.
Absence of IOS1 does not strengthen defense responses

The interaction phenotypes of the *ios1-1* mutant might be the consequence of either a loss of susceptibility, or a gain of resistance. We thus analyzed whether the mutation of *IOS1* led to increases in the activation of defense signaling pathways and immune responses. We used reverse-transcription quantitative PCR (RT-qPCR) to determine the expression of a set of genes that were previously described as being upregulated upon the onset of defense signaling. These genes code for FLG22-INDUCED RECEPTOR-LIKE KINASE 1 (FRK1), NDR1/HIN1-LIKE 10 (NHL10), PHYTOALEXIN DEFICIENT 4 (PAD4), and the transcription factors WRKY29, WRKY33, and WRKY70 (containing WRKY DNA-binding motifs). FRK1, NHL10, and WRKY29 are key components of PTI signaling pathways, and the genes encoding them are expressed at early time points upon perception of pathogen-associated molecular patterns (PAMPs) (Asai et al., 2002; He et al., 2006; Boudsocq et al., 2010). We did not detect statistically significant differences (*p* > 0.05) in the expression of *FRK1*, *NHL10*, and *WRKY29* between *Hpa*-infected Wt and *ios1-1* plants (Fig. 2A and B). The expression of *FRK1* follows the initial transient profile, which was previously reported for a compatible interaction with *Pseudomonas syringae* (He et al., 2006), and is then upregulated at later time points of *Hpa* infection in both the Wt and the mutant (Fig. 2A). Minor but significant differences (*p* < 0.05) between Wt and *ios1-1* plants were detectable in the transcriptional activation of *PAD4*, *WRKY33*, and *WRKY70* (Fig. 2A and B). An upregulation of these genes was observed during the early time points of interaction at 8-24 hours post inoculation (hpi). PAD4 is a central regulator in the generation of downstream disease resistance responses following effector-triggered activation of TIR-NB-LRR proteins, and is involved in salicylic acid (SA)-mediated signal transduction (Wiermer et al., 2005). WRKY33 integrates jasmonic acid (JA)- and ethylene-dependent defense pathways (Birkenbihl et al., 2012; Meng et al., 2013), whereas WRKY70 acts as a node of convergence for SA- and JA-dependent signaling events (Li et al., 2006). We therefore analyzed whether the mild increases in *PAD4*, *WRKY33*, and *WRKY70* expression affect the balance between SA-, JA-, and ethylene-dependent signaling in the mutant. To this end, we determined the influence of IOS1 on the transcriptional activation of SA, JA, and ethylene target genes in response to *Hpa* infection. *PR1a* expression reflects the activation of SA-mediated responses, whereas *PDF1.2* and *PR4* are marker genes for the JA- and ethylene-mediated defense signaling pathway in Arabidopsis (Robert-Seilaniantz et al., 2011). During *Hpa* infection, SA-dependent responses are the most
effective, while JA and ethylene-dependent responses do not generally play a major role
in defense against downy mildew (Glazebrook, 2005). Accordingly, we found a strong
induction of PR1a expression upon Hpa-infection, whereas PR1a transcripts were nearly
absent from water-treated controls. The observed induction by Hpa was not enhanced in
the ios1-1 background, but rather reduced, likely due to the lower infection density in the
mutant (Fig. 2C). Pathogen-responsive transcriptional activation of PR4 and PDF1.2
occurred to much lower levels than PR1a, and significant increases in the mutant
background were not detected, when compared to the Wt (cutoff: p<0.05). Similar to
PR1a, infection-responsive expression of PR4 was lower in ios1-1 (Fig. 2C) than in the
Wt.

We then analyzed whether reduced susceptibility of ios1-1 to Ec was
accompanied by increases in callose deposition. The amount and extent of callose
reflects the defense response of Arabidopsis towards the powdery mildew fungus
(Ellinger et al., 2013). We found no obvious differences between the Wt and the mutant,
and we could not observe an increased number or size of callose depositions around Ec
penetration sites in the ios1-1 mutant (Supplemental Fig. S3).

Although we cannot exclude that subtle changes in immune signaling pathways
are caused by the ios1-1 mutation, we conclude from our data that reduced susceptibility
of ios1-1 unlikely results from enhanced defense activation, but that it is rather due to the
lack of a host function, which is required for successful infection.

Absence of IOS1 confers hypersensitivity to abscisic acid

To gather more information on the physiological function of IOS1, we investigated
its expression during the Arabidopsis life cycle. We observed tissue-specific IOS1
expression in the radicle emerging from the testa, in the elongation zones of roots, and in
root cap border cells undergoing detachment (Fig. 3A-E). In hypocotyls, IOS1 expression
was detected only during etiolation in the dark (Fig. 3F and G). In cotyledons and leaves,
expression was restricted to cells surrounding stomata (Fig. 3H). In reproductive organs,
IOS1 expression was observed in the style after pollination, in the abscission zones of
sepals and petals, in the transmitting tract of developing fruits, and in the abscission
zones of mature siliques (Fig. 3I-O). In summary, IOS1 was predominantly expressed in
tissue with fates controlled by the phytohormone ABA. However, exogenous ABA
application to leaves did not activate the IOS1 promoter (Supplemental Fig. S4A), and
the elimination of ABA from roots with an inhibitor of carotenoid synthesis did not impair
**IOS1** expression (Supplemental Fig. S4B and C). IOS1 expression is therefore not upregulated by ABA.

Based on the **IOS1** expression profile, we investigated the possible involvement of the receptor kinase in ABA signaling. Exogenous ABA application to seeds and seedlings inhibits seed germination and cell elongation, respectively. We found that **ios1-1** mutant plants were significantly more sensitive to ABA-induced inhibition of both seed germination (Fig. 4A) and primary root elongation (Fig. 4B), when compared to plants from the Wt and the complemented mutant line, **ios1-1cp4**. ABA also modulates stomatal aperture and regulates foliar transpiration thus influencing the leaf surface temperature.

We investigated the effect of IOS1 on stomatal aperture, by subjecting leaves from Wt and mutant plants to thermographic measurements (Jones, 1999). We found that the rosette leaf temperatures of **ios1-1** were significantly higher under continuous light than those of the Wt, but similar to those of the ABA-hypersensitive **abh1** mutant (Hugouvieux et al., 2001; Fig. 4C). Since we could not distinguish the **ios1-1** mutant from Wt in terms of stomata morphology or density (Fig. 4D), we concluded that the higher leaf surface temperature of **ios1-1** resulted from limited pore aperture. The transition from darkness to light induces stomatal opening, which is inhibited by ABA (Mustilli et al., 2002). By contrast, the treatment of epidermal strips with the ATP-sensitive potassium channel inhibitor, glibenclamide, leads to the concerted inhibition of outward Cl⁻ and K⁺ channels in guard cells, inducing stomatal opening even in the dark (Leonhardt et al., 1999). We measured stomatal pore width on epidermal strips from Wt and mutant leaves treated with water or ABA in the dark before exposure to light. Alternatively, epidermal strips were treated with water and glibenclamide in the dark, and were then further incubated in the dark. We observed that ABA inhibited stomatal opening in the light by about 0.5 µm on epidermal strips from Wt plants, but by about 2.5 µm on those from the **ios1-1** mutant. Stomatal opening was therefore more sensitive to ABA in the mutant than in the Wt. Glibenclamide treatment induced stomatal opening in the dark by about 1 µm in both the Wt and the mutant (Fig. 4E), suggesting that the **ios1-1** mutation had no effect on ABA-responsive ion channels. We then compared the stomatal conductance of leaves in response to different ABA concentrations. In the absence of ABA, stomatal conductance did not differ significantly between mutant and Wt plants under our experimental conditions. Increasing ABA concentrations decreased conductance in all lines (Fig. 4F). This effect was significantly stronger in the **ios1-1** mutant than in the Wt or the **ios1-1cp4**
line. Taken together, our findings show that a loss of IOS1 confers ABA hypersensitivity to Arabidopsis.

**ABA signaling interferes with infection by downy mildew, and is attenuated by IOS1**

We analyzed the ABA contents in non-inoculated and *Hpa*-infected cotyledons from Wt plants, the *ios1-1* mutant, and the complemented mutant *ios1-1cp4*, and did not find significant differences in ABA levels between genotypes (Supplemental Fig. S5). These data suggest that IOS1 interferes with ABA signaling or the onset of responses, rather than with the accumulation of the hormone, and that ABA-hypersensitivity of *ios1-1* derives from the absence of a negative regulatory element in ABA signaling. We thus analyzed the transcriptional induction of genes coding for COLD REGULATED (COR) and RESISTANCE TO DESSICATION (RD) proteins in cotyledons from Wt and *ios1-1* mutant plants at different time points after water treatment or *Hpa* inoculation. The transcriptional upregulation of *COR* and *RD* genes conventionally reflects activated ABA signaling in Arabidopsis (Guo et al., 2002). We found that *COR15A*, *COR15B* and *RD29A* were transiently activated in the control situation, i.e. in water-treated cotyledons of plants that were grown at 20°C and transferred to the inoculation temperature of 16°C. Transcription of these genes was enhanced in *ios1-1* at all time points, thus confirming ABA-hypersensitivity of the mutant, and reduced upon inoculation with *Hpa* in both genotypes (Fig. 5A). The reduction in *COR/RD* gene expression upon infection was less pronounced in *ios1-1* than in the Wt, particularly during the first day of infection. We found that *COR15A* and *COR15B* were transiently induced upon exogenous application of ABA to cotyledons, and that transcript accumulation peaked around 2 to 4 hours after spray treatment of the Wt or of *ios1-1cp4*. In ABA-treated *ios1-1* mutant plants, a maximum of *COR* gene transcripts accumulated already 1 h after ABA application (Supplemental Fig. S6). IOS1 thus appears to interfere with the timing of ABA signaling, rather than with its amplitude.

Among the *COR/RD* marker genes that were analyzed by RT-qPCR for infection-related regulation in the absence or presence of IOS1, we found weak differences in *RD29B* transcript levels. We thus used an experimental approach that allowed measuring the cumulative, ABA-dependent transcriptional activation of this gene over 3 days of treatment. We generated *ios1-1* mutant and Wt lines harboring a fusion between the *RD29B* promoter (Christmann et al., 2005) and the *uid A* gene (Jefferson et al., 1987), which allowed us to measure the activity of the stable GUS reporter as a readout
for *in planta* and *in vitro* studies. We stimulated ABA signaling in water-treated and *Hpa*-infected cotyledons, either by applying ABA, or by lowering relative humidity to stimulate drought responses (Uno et al., 2000). Both treatments enhanced *RD29B* promoter activity and stimulated synthesis of the GUS reporter protein in uninfected cotyledons from both Wt and *ios1-1* plants. Reporter activity was substantially reduced in *Hpa*-infected cotyledons of the Wt, but not in *Hpa*-infected cotyledons of the *ios1-1* mutant (Fig. 5B; Supplemental Fig. S7). To obtain quantitative data, we measured GUS activity under the different experimental conditions. In the Wt background, infection with *Hpa* decreased GUS by up to 60%. No downregulation of ABA-sensitive reporter gene expression occurred in the *ios1-1* mutant background, and GUS activity was comparable in the presence and absence of infection (Supplemental Fig. S7B).

Our findings suggest that IOS1 downregulates ABA signaling, thereby promoting disease progression in downy mildew-infected tissue (Supplemental Fig. S8). This implies that ABA signaling impedes the infection process, and that its downregulation promotes disease. Consistent with this hypothesis, we found that exogenous ABA application to cotyledons significantly interfered with downy mildew disease on the Wt and the complemented mutant, whereas it did not further reduce sporulation on *ios1-1* (Fig. 5C). In further support of this hypothesis, we noted that ABA-insensitive (*abi1-1*; Koornneef et al., 1984) and hypersensitive (*abh1*) signaling mutants were significantly more susceptible and more resistant to *Hpa* infection, respectively (Fig. 5D).

**DISCUSSION AND CONCLUSION**

Our findings identify IOS1 as a novel element in the network regulating ABA responses. IOS1 expression is specific to particular tissues or developmental stages exhibiting a tight control of ABA responses (Fig. 3), and becomes induced in response to infection with filamentous, hemibiotrophic or biotrophic pathogens (Fig. 1). Our data thus suggest that these pathogens benefit from IOS1-mediated downregulation of ABA signaling to accomplish their infection cycle. The role of ABA in plant-microbe interactions is ambiguous, but seems to be dependent on the lifestyle of infecting pathogens, the plant tissue, and the time point of infection (Ton et al., 2009). The Arabidopsis *abi1-1* ABA signaling mutant was previously shown to be susceptible to *Hpa*, but more resistant to *P. syringae*, thus indicating that ABA signaling promotes the multiplication of the bacterium, but not the development of *Hpa* in plant tissues (Mohr and Cahill, 2003). We show that susceptibility to downy mildew is increased in cotyledons of the signaling mutant *abi1-1*, supporting that *P. syringae* and *Hpa* require
ABA signaling in Arabidopsis in an opposite manner during the initial infection process. Consistent with this, we found the ABA-hypersensitive mutant abh1 more resistant to Hpa-infection, whereas the opposite effect was observed when this mutant interacted with P. syringae (de Torres-Zabala et al., 2007). Since our data show that neither the IOS1 genotype nor Hpa infection had an influence on ABA levels in cotyledons (Supplemental Fig. S5), we conclude that IOS1 interferes with ABA signaling rather than with its accumulation. Although ios1-1 has an ABA hypersensitive-like phenotype, susceptibility to downy mildew is less strongly reduced in ios1-1 than in abh1. We previously showed that IOS1 is encoded by a gene that gave rise to a cluster of 11 IOS1-like MLD-RLKs through successive gene duplications (Hok et al., 2011). Three other genes from the cluster were found to be co-regulated with IOS1 during downy mildew infection (Hok et al., 2011). The weaker interaction phenotype of the ios1-1 mutant, when compared to abh1, might reflect that other members of the cluster participate in the downregulation of ABA signaling, and co-operate in disease promotion.

The oomycete pathogens studied here sporulate through stomata, and Pp also employs stomata to re-enter leaf tissues (see Supplemental Fig. S8). Increasing stomatal opening due to a downregulation of ABA signaling would thus favor the entry and exit of these pathogens. However, powdery mildew infection is largely independent of stomatal opening, as the fungus feeds on epidermal cells and does not enter the leaf tissue. Furthermore, IOS1 appears to play a predominant role during the early stages of infection, whereas the sporulation of Hpa through open stomata characterizes the end of the disease cycle. Induced stomatal opening might thus favor other parameters affecting biotrophic infection, such as the increase in transpiration and water loss from leaf cells. The resulting decrease in leaf cell turgor pressure might induce phloem cells with a higher turgor pressure to discharge their metabolites into the leaf cells, which would potentially become sinks for nutrients that might then become available to the feeding structures of biotrophic pathogens. Independent of the effects on stomata, ABA influences stress responses as well as aging of plant tissues (Lim et al., 2007). IOS1-mediated downregulation of ABA signaling results in the activation of COR and RD gene expression. These genes are transiently expressed during the “cooling” responses of Arabidopsis (Wang and Hua, 2009), which we likely induced in our experimental setup, and are also expressed during senescence of plant tissues (Yang et al., 2011). The downregulation of COR/RD genes upon infection might thus indicate that biotrophs
attenuate stress-associated senescence programs of the host cells for successful infection.

ABA also interferes with signaling pathways involving the defense hormones SA, JA, and ethylene. One would expect that an interference of IOS1 with defense hormone signaling upon *Hpa* infection would influence the expression of defense-related genes that are controlled by these hormones. However, the conventional marker genes *PR1a*, *PR4*, and *PDF1.2* were not more responsive in *ios1-1* upon *Hpa* infection, when compared to the Wt. By contrast, genes encoding the defense regulators WRKY33, WRKY70, and PAD4 were upregulated to some extent in *ios1-1* at the early stages of infection. WRKY33 seems also to be involved in the regulation of thermotolerance (Li et al., 2011), and WRKY70 was recently described as a negative regulator of stomatal closure and osmotic stress tolerance in Arabidopsis (Li et al., 2013). PAD4 is essential in effector-triggered immune signaling, but it is also required for the downregulation of ABA signaling (Kim et al., 2011). These genes are thus involved both in defense, and in the negative regulation of ABA signaling. Their activation in *ios1-1* might reflect the existence of a feedback loop, which counteracts excessive ABA signaling. We cannot exclude that a subtly nuanced crosstalk between ABA and defense hormones contributes to the observed interaction phenotypes of the *ios1-1* mutant. But taken together, our findings indicate a loss of susceptibility to (hemi)biotrophic filamentous pathogens rather than a gain of defense in plants that lack IOS1.

IOS1 appears to have a multifaceted role in the interaction of Arabidopsis with microbes, depending on the kind of the infecting pathogen. Our findings determine IOS1 as a plant factor promoting infection by filamentous (hemi-)biotrophs, and as a negative regulatory element of ABA signaling. By contrast, IOS1 plays a critical role in the onset of PTI during the interaction of Arabidopsis with *P. syringae* (Chen et al., 2014). It associates with the recognition receptors for bacterial PAMPs, FLS2 and EFR, and their co-receptor BAK1. The absence of IOS1 in mutant plants confers an attenuated PTI response and increased susceptibility to the bacteria. In this interaction, IOS1 acts as a positive regulatory element in PTI upstream of MAP kinase signaling. Consequently, both the expression of PTI marker genes (such as *FRK1*) and callose deposition were attenuated in the absence of IOS1 upon bacterial perception. During the interaction with filamentous (hemi-)biotrophs, however, IOS1 seems to be engaged in a PTI-independent signaling pathway, as we found that the induction of genes involved in early defense signaling (*FRK1*, *NHL10*, and *WRKY29*) upon *Hpa* infection, and callose deposition in...
the interaction with powdery mildew did not change in ios1-1, when compared to the Wt plants. Chen and coworkers (2014) also found that IOS1 did not interfere with infection by the necrotrophic fungal pathogen, *Botrytis cinerea*, further supporting a pathogen-dependent role of IOS1. It has to be noted that three allelic mutants, ios1-1, ios1-2, and ios1-3, are available. The mutant ios1-1 is a full knock-out, whereas both ios1-2 and ios1-3 produce transcripts that probably translate for IOS1 proteins with a truncated kinase domain (Chen et al., 2014, and Supplemental Fig. S9). In the interaction with *P. syringae*, ios1-1, ios1-2, and ios1-3 showed similar PTI defects, and it was also shown that IOS1 requires an active kinase for the association with FLS2 and BAK1, and for the concomitant activation of the PTI response after challenge with *P. syringae* (Chen et al., 2014). By contrast, signaling is probably independent of the IOS1 kinase activity upon infection with *Hpa*, because in our hands only ios1-1, but not ios1-2 or ios1-3, was hypersensitive to ABA and more resistant to *Hpa* (data not shown). We suppose that IOS1 may associate with other protein(s) that assure(s) the downstream signaling events during the interaction with *Hpa* and other filamentous (hemi-)biotrophs, independent of the intrinsic IOS1 kinase domain. Interestingly, a recent large-scale analysis of the membrane protein interactome of Arabidopsis indicated that IOS1 interacts with a protein phosphatase 2C (Jones et al., 2014), which belongs to the ABI1-related PP2C family (Schweighofer et al., 2004). This finding requires confirmation, but it may indicate that IOS1 downregulates ABA signaling at the PP2C node, where the ABA signal is transduced through the inhibition of PP2Cs by activated PYR/PYL/RCAR receptors (Cutler et al., 2010). The same screen revealed CRK50, a cystein-rich RLK, as another protein that potentially interacts with IOS1 (Jones et al., 2014). This may further support that IOS1 employs different modes of signaling for the activation of PTI in the interaction with *P. syringae*, and for the downregulation of ABA upon infection with *Hpa*.

MLD-RLKs constitute a large protein family in Arabidopsis, but functional data have only recently become available for some of the family members. The MLD-RLKs described to date control several processes involved in reproduction. MLD-RLKs from the OUTGROWTH-ASSOCIATED PROTEIN KINASE (OAK) cluster control the combination quality of intraspecific hybrids after fertilization (Smith et al., 2011). THESEUS1 (THE1) and HERKULES1 (HERK1) govern cell elongation in flower stalks (Guo et al., 2009). ANXUR (ANX1 and ANX2) and FER coordinate the behavior of male and female reproductive organs, respectively, during pollen tube perception in fertilization (Boisson-Dernier et al., 2009; Miyazaki et al., 2009). FER is responsible for
egg cell polarization, and fer mutants are particularly resistant to powdery mildew fungi (Kessler et al., 2010). FER has recently been shown to downregulate ABA signaling (Yu et al., 2012), and it has been suggested that tip-growing hyphae from the fungus resemble progressing pollen tubes (Kessler et al., 2010). In this scenario, the hyphae make use of FER, as a conserved component, to polarize host cells before establishing haustoria, and to downregulate ABA signaling to facilitate disease progression. The functional analogy of IOS1 with FER (negative regulation of ABA signaling), and its' structural similarity with SYMRK, suggest that IOS1 is one of the conserved elements that evolved in plants for the detection of beneficial filaments, and which may have since been exploited by pathogens for the establishment of disease.

In conclusion, we show that IOS1 negatively regulates ABA signaling, and that an absence of the MLD-RLK confers ABA hypersensitivity to Arabidopsis. Our findings strongly suggest that mildews exploit the receptor to downregulate ABA signaling in the host upon infection. IOS1 thus appears to pivot the outcome of interactions between Arabidopsis and the biotic environment, as it both primes PTI to P. syringae, and supports the infection success of filamentous biotrophs.
MATERIALS AND METHODS

Plant material.

The *Arabidopsis thaliana* mutants *ios1-1* (Hok et al., 2011), *abh1* (Hugouvieux et al., 2001), *abi1-1* (Korneef et al., 1984), and the complemented *ios1-1cp4* line (Hok et al., 2011) were described. A line harboring the *pRD29B::GUS* reporter (Christmann et al., 2005) in the Ler background was crossed with *ios1-1*, and lines homozygous for both the mutation and the reporter transgene were used for analysis.

Plant growth conditions and treatments.

If not otherwise stated, plants were grown in growth cabinets at 20°C with a 12-h photoperiod (Hok et al., 2011). For germination assays, seeds were sown on Gamborg B5 medium including vitamins (Duchefa, Haarlem, The Netherlands), which was complemented with 1% sucrose and ABA (± racemate containing 50% active ABA, Sigma-Aldrich A1049). For growth assays, seeds were sown without ABA, and transferred to medium containing 10 µM (±)-ABA 1 week later. Root elongation was measured from this time point. Alternatively, one week old plantlets from the reporter line were transferred to medium containing Fluridon (Sigma-Aldrich 45511). The responsiveness of *COR* genes to exogenous ABA application was analyzed after spraying water, or (±)-ABA at 0.5 and 5 µM onto 8 d-old seedlings, and further incubation at 20°C in the light in water-saturated atmosphere. For analyzing the influence of exogenously applied ABA on the interaction with *Hpa*, 7 d-old seedlings were sprayed twice with water or 10 µM (±)-ABA, 6 h prior to inoculation, and 24 h post-inoculation with conidiospores. To stimulate RD29B expression, plants were sprayed with a solution containing 10 µM (±)-ABA, 0.1% DMSO and 0.005% Silwett, one day after inoculation with *Hpa* or treatment with water. Alternatively, plants inoculated or not by *Hpa* were placed in a Superdry SD 302-21 cabinet (Totech, Dronten, The Netherlands) at 15% relative humidity, 2 days after inoculation.

Pathogen assays.

Interaction studies with *Hpa* and *Pp* were performed and analyzed according to (Hok et al., 2011) and (Attard et al., 2010), respectively. Conidia from *Ec* were sprayed on five week-old plants at an inoculation density of 3-5 conidia mm⁻². Powdery mildew symptoms were examined 7 days after inoculation. For conidiophore production, leaves were fixed 5 days after inoculation, and fungal structures were stained with acetic ink.
Inoculations with *R. solanacearum* strain GMI1000, and the scoring of disease symptoms were performed according to (Hirsch et al., 2002).

**Determination of ABA concentrations in plant tissues**

Lyophilized plant material (100 mg fresh weight equivalent per sample) was homogenized. Each sample was analyzed as two technical and three biological replicates. [2H₆]-ABA (Plant Biotechnology Institute, National Research Council of Canada, Saskatoon, Canada) Further sample preparation was performed according to Meixner et al. (2005) with some modifications. After centrifugation of the samples at 10,000*g* for 10 min, the supernatant was removed and evaporated to dryness under a stream of N₂. The residue was resuspended in 200 µL methanol, centrifuged again under the same conditions, the supernatant removed and placed in a glass vial. The methanol was evaporated under a stream of N₂ and the sample resuspended in methanol.

Methylation was performed by adding equal sample amounts of a 1:10 diluted solution (in diethylether) of trimethylsilyldiazomethane solution (Sigma-Aldrich, Germany) for 30 min at room temperature. The mixture was then evaporated and resuspended in 50 µL ethyl acetate for GC-MS analysis.

GC-MS analysis was carried out on a Varian Saturn 2100 ion-trap mass spectrometer using electron impact ionization at 70 eV, connected to a Varian CP-3900 gas chromatograph equipped with a CP-8400 autosampler (Varian, Walnut Creek, CA, USA). For the analysis 1 µL of the methylated sample was injected in the splitless mode (splitter opening 1:100 after 1 min) onto a Phenomenex (Aschaffenburg, Germany) ZB-5 column (30 m x 0.25 mm x 0.25 µm) using He carrier gas at 1 mL min⁻¹. Injector temperature was 250°C and the temperature program was 60°C for 1 min, followed by an increase of 25°C min⁻¹ to 180°C, 5°C min⁻¹ to 250°C, 25°C min⁻¹ to 280°C, then 5 min isothermically at 280°C. For higher sensitivity, the µSIS mode (Varian Manual; Wells and Huston, 1995) was used. The endogenous hormone concentrations were calculated by the principles of isotope dilution (at m/z 190/194 (endogenous and labeled standard; note that during fragmentation of ABA two deuterium are lost) for methylated ABA (Walker-Simmons et al., 2000).

**Detection of reporter gene expression.**

Samples were analyzed by confocal microscopy for fluorescence of the GFP reporter. GUS activity was stained histochemically with X-Gluc (Eurogentec, Angers, France), and analyzed with a Zeiss Axioplan 2 microscope (Carl Zeiss, Jena, Germany) using transmission light optics. For localization in thin-sectioned tissues, X-Guc-stained...
cotyledons were fixed in 2% glutaraldehyde in 50 mM PIPES, pH 6.9, dehydrated, and embedded in Technovit 7100 (Heraeus Kulzer) as described by the manufacturer. Embedded tissues were sectioned (6 µm) and mounted in Depex (Sigma-Aldrich). Microscopy was performed on the Zeiss Axioplan 2 using dark-field optics. *In vitro* GUS activity was determined through the enzymatic conversion of 4-methylumbelliferyl-glucuronide (MUG; Jefferson et al., 1987). Fluorescence of generated methylumbelliferone (MU) was determined with an automated Xenius fluorimeter (SAFAS, Monaco) under continuous agitation (600 V, ex 350 nm, em 450 nm, bandwith 10 nm). Specific enzymatic activity was calculated according to a calibration curve established with commercial MU (Sigma-Aldrich).

**Confocal microscopy.**

Optical sections were obtained using an inverted confocal microscope (model LSM 510 META; Carl Zeiss, Jena, Germany) equipped with an Argon ion and HeNe laser as excitation source. GFP fluorescence emission in samples was detected after excitation at 488 nm through a 505-530 nm band-pass emission filter.

**RT-PCR.**

RNA was extracted from 50 mg of seedlings with the Isolate II RNA Plant Kit (Bioline GmbH, Luckenwalde, Germany). Reverse transcription, qPCR, and data analysis were performed according to (Hok et al., 2011).–For primer sequences, see Supplemental Table S1.

**Thermography.**

Plants were grown for 4 weeks at 20°C with an 8-h photoperiod. Before Thermography, plants were exposed for 48 h to continuous light at room temperature under low relative humidity. In 3 experimental repetitions, infrared recordings were performed with a Thermacam PM250 (Inframetrics, FLIR Systems, North Billerica, MA, USA) on at least 20 positions per rosette for 8 different plants per line.

**Measurement of stomatal density, aperture, and conductance.**

Epidermal peels of leaves from 4 week-old plants were prepared on a microscope slide with medical adhesive Telesis V (Premiere Products, Pacoima, CA). Stomata were allowed to close for 2.5 h in the dark in a medium containing 10 mM MES, 10 mM KCl, and 7.5 mM imminodiacetic acid at pH 6.2, and were then either incubated for 3 h in white light in the same medium containing 50 µM (±)-ABA, or kept in the dark for 3 h after
addition of 10 µM glibenclamide (Enzo Life Sciences, Villeurbanne, France). All peels were analyzed with an Axioplan 2 microscope equipped with an Axiocam camera (Carl Zeiss, Jena, Germany). Stomatal density and -aperture were determined with the Zeiss Axiovision digital image-processing software, version 4.4. Stomatal conductance g(H₂O) was measured with leaves of 6 week-old short-day grown plants using a GFS-3000 infrared gas analyzer (Walz, Effeltrich, Germany). Assimilation rate, transpiration rate and stomatal conductance were calculated using the GFSwin v2.0 software (Walz, Effeltrich, Germany). To analyze ABA-dependent stomatal closure, leaves were detached, transferred to 2 mL of water, and adapted for 10 min to light (200 µEm⁻² s⁻¹), temperature (22°C) and CO₂ (350 ppm). Petioles were then transferred to different ABA concentrations, and gas exchange was recorded in a 3 cm² cell at 200 µEm⁻² s⁻¹, 22 ºC, and 13,000 ppm H₂O (56% relative humidity) until stomatal aperture reached steady state at about 25-40 min after transfer. To increase reproducibility, measurements were conducted only during the first half of the light period.

Sequence data from this article, and details for Arabidopsis loci can be found at the GenBank/EMBL data libraries, and The Arabidopsis Information Resource under accession numbers At1g51800 (IOS1), At2g19190 (FRK1), At2g35980 (NHL10), At2g38470 (WRKY33), At3g52430 (PAD4), At2g14610 (PR1a), At2g26020 (PDF1-2), At3g04720 (PR4), At4g23550 (WRKY29), At3g56400 (WRKY70), At5g52310 (RD29A), AT5G52300 (RD29B), At2g42530 (COR15B), At2g42540 (COR15A), At5g10790 (UBP22), At5g11770 (NADH-ubiquinone oxidoreductase 20 kDa subunit), and At5g62050 (OXA1).

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SUPPLEMENTAL DATA

The following materials are available in the online version of this article.

Supplemental Figure S1. IOS1 affects powdery mildew disease, but not bacterial wilt.
Supplemental Figure S2. *ios1-1* mutants have no aberrant developmental phenotypes.

Supplemental Figure S3. The *ios1-1* mutation does not affect callose deposition in powdery mildew-infected leaves.

Supplemental Figure S4. Abscisic acid (ABA) does not induce *IOS1* expression.

Supplemental Figure S5. ABA levels in cotyledons from Arabidopsis plants.

Supplemental Figure S6. ABA-induced, transient expression of *COR* genes is accelerated in the *ios1-1* mutant.

Supplemental Figure S7. ABA-sensitive reporter gene expression is downregulated in *Hpa*-inoculated Wt, but not in *ios1-1*.

Supplemental Figure S8. Proposed role of IOS1 during infection with filamentous (hemi-)biotrophs.

Supplemental Figure S9. Transcripts produced by *ios1* mutants.

Supplemental Table S1. Accession numbers, names and primer sequences for genes analyzed in this study.
LITERATURE CITED

Albrecht C, Boutrot F, Segonzac C, Schwessinger B, Gimenez-Ibanez S, Chinchilla D, Rathjen JP, de Vries SC, Zipfel C (2012) Brassinosteroids inhibit pathogen-associated molecular pattern-triggered immune signaling independent of the receptor kinase BAK1. Proc Natl Acad Sci USA 109: 303-308

Asai T, Tena G, Plotnikova J, Willmann MR, Chiu WL, Gomez-Gomez L, Boller T, Ausubel FM, Sheen J (2002) MAP kinase signaling cascade in Arabidopsis innate immunity. Nature 415: 977-983

Attard A, Gourgues M, Callemeyn-Torre N, Keller H (2010) The immediate activation of defense responses in Arabidopsis roots is not sufficient to prevent Phytophthora parasitica infection. New Phytol 187: 449-460

Bai L, Zhang G, Zhou Y, Zhang Z, Wang W, Du Y, Wu Z, Song CP (2009) Plasma membrane-associated proline-rich extensin-like receptor kinase 4, a novel regulator of Ca^{2+} signalling, is required for abscisic acid responses in Arabidopsis thaliana. Plant J 60: 314-327

Belkhadir Y, Jaillais Y, Epple P, Balsemão-Pires E, Dangl JL, Chory J (2012) Brassinosteroids modulate the efficiency of plant immune responses to microbe-associated molecular patterns. Proc Natl Acad Sci USA 109: 297-302

Birkenbihl RP, Diezel C, Somssich IE (2012) Arabidopsis WRKY33 is a key transcriptional regulator of hormonal and metabolic responses toward Botrytis cinerea infection. Plant Physiol 159: 266-285

Boisson-Dernier A, Roy S, Kritsas K, Grobei MA, Jaciubek M, Schroeder JL, Grossniklaus U (2009) Disruption of the pollen-expressed FERONIA homologs ANXUR1 and ANXUR2 triggers pollen tube discharge. Development 136: 3279-3288

Boudsocq M, Willmann MR, McCormack M, Lee H, Shan L, He P, Bush J, Cheng SH, Sheen J (2010) Differential innate immune signalling via Ca^{2+} sensor protein kinases. Nature 464: 418-422

Capoen W, Goormachtig S, De Rycke R, Schroeyers K, Holsters, M (2005) SrSymRK, a plant receptor essential for symbiosome formation. Proc Natl Acad Sci USA 102: 10369-10374
Chen CW, Panzeri D, Yeh YH, Kadota Y, Huang PY, Tao CN, Roux M, Chien SC, Chin TC, Chu PW, Zipfel C, Zimmerli L (2014) The Arabidopsis malectin-like leucine-rich repeat receptor-like kinase IOS1 associates with the pattern recognition receptors FLS2 and EFR and is critical for priming of pattern-triggered immunity. Plant Cell 26: 3201-3219.

Chen Y, Hu D, Yabe R, Tateno H, Qin SY, Matsumoto N, Hirabayashi J, Yamamoto K (2011) Role of malectin in Glc$_2$Man$_9$GlcNAc$_2$-dependent quality control of $\alpha$1-antitrypsin. Mol Biol Cell 22: 3559-3570

Chinchilla D, Zipfel C, Robatzek S, Kemmerling B, Nürnberger T, Jones JD, Felix G, Boller T (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. Nature 448: 497-500

Chinchilla D, Shan L, He P, de Vries S, Kemmerling B (2009) One for all: the receptor-associated kinase BAK1. Trends Plant Sci 14: 535-541

Christmann A, Hoffmann T, Teplova I, Grill E, Müller A (2005) Generation of active pools of abscisic acid revealed by in vivo imaging of water-stressed Arabidopsis. Plant Physiol 137: 209-219

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J 16: 735–743

Cohen JD, Baldi BG, Slovin JP (1986) $^{13}$C$_6$-[benzene ring]-indole-3-acetic acid: A new internal standard for quantitative mass spectral analysis of Indole-3-Acetic Acid in plants. Plant Physiol 80: 14-19

Cutler SR, Rodriguez PL, Finkelstein RR, Abrams SR (2010) Abscisic acid: emergence of a core signaling network. Annu Rev Plant Biol 61: 651-679

de Torres-Zabala M, Truman W, Bennett MH, Lafforgue G, Mansfield JW, Rodriguez Egea P, Bögre L, Grant M (2007) Pseudomonas syringae pv. tomato hijacks the Arabidopsis abscisic acid signalling pathway to cause disease. EMBO J 26:1434-1443

Ellinger D, Naumann M, Falter C, Zwikowics C, Jamrow T, Manisseri C, Somerville SC, Voigt CA (2013) Elevated early callose deposition results in complete penetration resistance to powdery mildew in Arabidopsis. Plant Physiol 161: 1433-1444

Glaizebrook J (2005) Contrasting mechanisms of defense against biotrophic and...
necrotrophic pathogens. Annu Rev Phytopathol 43: 205-227

Guo H, Li L, Ye H, Yu X, Algreen A, Yin Y (2009) Three related receptor-like kinases are required for optimal cell elongation in Arabidopsis thaliana. Proc Natl Acad Sci USA 106: 7648-7653

Guo Y, Xiong L, Song CP, Gong D, Halfter U, Zhu JK (2002) A calcium sensor and its interacting protein kinase are global regulators of abscisic acid signaling in Arabidopsis. Dev Cell 3: 233-244

He P, Shan L, Lin NC, Martin GB, Kemmerling B, Nürnberger T, Sheen J (2006) Specific bacterial suppressors of MAMP signaling upstream of MAPKKK in Arabidopsis innate immunity. Cell 125: 563-575

Hernández-Blanco C, Feng DX, Hu J, Sánchez-Vallet A, Deslandes L, Llorente F, Berrocal-Lobo M, Keller H, Barlet X, Sánchez-Rodríguez C, et al (2007) Impairment of cellulose synthases required for Arabidopsis secondary cell wall formation enhances disease resistance. Plant Cell 19: 890-903

Hirsch J, Deslandes L, Feng DX, Balagué C, Marco Y (2002) Delayed symptom development in ein2-1, an Arabidopsis ethylene-insensitive mutant, in response to bacterial wilt caused by Ralstonia solanacearum. Phytopathology 92: 1142-1148

Hoefle C, Huesmann C, Schultheiss H, Börnke F, Hensel G, Kumlehn J, Hückelhoven R (2011) A barley ROP GTPase ACTIVATING PROTEIN associates with microtubules and regulates entry of the barley powdery mildew fungus into leaf epidermal cells. Plant Cell 23: 2422-2439

Hok S, Danchin EG, Allasia V, Panabières F, Attard A, Keller H (2011) An Arabidopsis (malectin-like) leucine-rich repeat receptor-like kinase contributes to downy mildew disease. Plant Cell Environ 34: 1944-1957

Hua J, Meyerowitz EM (1998) Ethylene responses are negatively regulated by a receptor gene family in Arabidopsis thaliana. Cell 94: 261-271

Hückelhoven R, Kogel KH (1998) Tissue-specific superoxide generation at interaction sites in resistant and susceptible near-isogenic barley lines attacked by the powdery mildew fungus (Erysiphe graminis f. sp. hordei). Mol Plant-Microbe Interact 11: 292-300
Hugouvieux V, Kwak JM, Schroeder JI (2001) An mRNA cap binding protein, ABH1, modulates early abscisic acid signal transduction in Arabidopsis. Cell 106: 477-487

Igarashi D, Tsuda K, Katagiri F (2012) The peptide growth factor, phytosulfokine, attenuates pattern-triggered immunity. Plant J 71: 194-204

Inoue T, Higuchi M, Hashimoto Y, Seki M, Kobayashi M, Kato T, Tabata S, Shinozaki K, Kakimoto T (2001) Identification of CRE1 as a cytokinin receptor from Arabidopsis. Nature 409: 1060-1063

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: Beta-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J 6: 3901–3907

Jones HG (1999) Use of thermography for quantitative studies of spatial and temporal variation of stomatal conductance over leaf surfaces. Plant Cell Eviron 22: 1043–1055

Jones, AM, Xuan Y, Xu M, Wang RS, Ho CH, Lalonde S, You CH, Sardi MI, Parsa SA, Smith-Valle E, et al (2014) Border control - a membrane-linked interactome of Arabidopsis. Science 344: 711-716.

Karimi M, Inze D, Depicker A (2002) GATEWAY vectors for Agrobacterium-mediated plant transformation. Trends Plant Sci 7: 193–195

Kessler SA, Shimosato-Asano H, Keinath NF, Wuest SE, Ingram G, Panstruga R, Grossniklaus U (2010) Conserved molecular components for pollen tube reception and fungal invasion. Science 330: 968-971

Kim TH, Hauser F, Ha T, Xue S, Böhmer M, Nishimura N, Munemasa S, Hubbard K, Peine N, Lee BH, et al (2011) Chemical genetics reveals negative regulation of abscisic acid signaling by a plant immune response pathway. Curr Biol 21: 990-997

Koornneef M, Reuling G, Karssen CM (1984) The isolation and characterization of abscisic acid-insensitive mutants of Arabidopsis thaliana. Physiol Plant 61: 377–383

Kosuta S, Held M, Hossain MS, Morieri G, Macgillivary A, Johansen C, Antolín-Llovera M, Parniske M, Oldroyd GE, Downie AJ, et al (2011) Lotus japonicus symRK-14 uncouples the cortical and epidermal symbiotic program. Plant J 67: 929-940

Leonhardt N, Vavasseur A, Forestier C (1999) ATP binding cassette modulators control abscisic acid-regulated slow anion channels in guard cells. Plant Cell 11: 1141–1152
Li J, Besseau S, Törönen P, Sipari N, Kollist H, Holm L, Palva ET (2013) Defense-related transcription factors WRKY70 and WRKY54 modulate osmotic stress tolerance by regulating stomatal aperture in *Arabidopsis*. New Phytol 200: 457-472

Li J, Brader G, Kariola T, Palva ET (2006) WRKY70 modulates the selection of signaling pathways in plant defense. Plant J 46: 477-491

Li S, Fu Q, Chen L, Huang W, Yu D (2011) *Arabidopsis thaliana* WRKY25, WRKY26, and WRKY33 coordinate induction of plant thermotolerance. Planta 233: 1237-1252

Libault M, Wan J, Czechowski T, Udvardi M, Stacey G (2007) Identification of 118 *Arabidopsis* transcription factor and 30 ubiquitin-ligase genes responding to chitin, a plant-defense elicitor. Mol Plant Microbe Interact 20: 900-911

Lim PO, Kim HJ, Nam HG (2007) Leaf senescence. Annu Rev Plant Biol 58: 115–136

Lindner H, Müller LM, Boisson-Dernier A, Grossniklaus U (2012) CrRLK1L receptor-like kinases: not just another brick in the wall. Curr Opin Plant Biol 15: 659-669

Liu X, Yue Y, Li B, Nie Y, Li W, Wu WH, Ma L (2007) A G protein-coupled receptor is a plasma membrane receptor for the plant hormone abscisic acid. Science 315: 1712-1716

Markmann K, Giczey G, Parniske M (2008) Functional adaptation of a plant receptor-kinase paved the way for the evolution of intracellular root symbioses with bacteria. PLoS Biol 6: e68

Matsubayashi Y, Ogawa M, Morita A, Sakagami Y (2002) An LRR receptor kinase involved in perception of a peptide plant hormone, phytosulfokine. Science 296: 1470-1472

Meixner C, Ludwig-Müller J, Miersch O, Gresshoff P, Staehelin C, Vierheilig H (2005) Lack of mycorrhizal autoregulation and phytohormonal changes in the supernodulating soybean mutant *nts1007*. Planta 222: 709-715

Meng X, Xu J, He Y, Yang KY, Mordorski B, Liu Y, Zhang S (2013) Phosphorylation of an ERF transcription factor by *Arabidopsis* MPK3/MPK6 regulates plant defense gene induction and fungal resistance. Plant Cell 25: 1126-1142

Miyazaki S, Murata T, Sakurai-Ozato N, Kubo M, Demura T, Fukuda H, Hasebe M (2009) ANXUR1 and 2, sister genes to FERONIA/SIRENE, are male factors for coordinated fertilization. Curr Biol 9: 1327-1331
Mohr PG, Cahill DM (2003) Abscisic acid influences the susceptibility of *Arabidopsis thaliana* to *Pseudomonas syringae* pv. tomato and *Peronospora parasitica*. Funct Plant Biol 30: 461-469

Mosher S, Seybold H, Rodriguez P, Stahl M, Davies KA, Dayaratne S, Morillo SA, Wierzba M, Favery B, Keller H, et al (2013) The tyrosine-sulfated peptide receptors PSKR1 and PSY1R modify the immunity of *Arabidopsis* to biotrophic and necrotrophic pathogens in an antagonistic manner. Plant J 73: 469-482

Motose H, Iwamoto K, Endo S, Demura T, Sakagami Y, Matsubayashi Y, Moore KL, Fukuda H (2009) Involvement of phytosulfokine in the attenuation of stress response during the transdifferentiation of zinnia mesophyll cells into tracheary elements. Plant Physiol 150: 437-447

Mustilli AC, Merlot S, Vavasseur A, Fenzi F, Giraudat J (2002) *Arabidopsis* OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. Plant Cell 14: 3089-3099

Nelson BK, Cai X, Nebenführ A (2007) A multicolored set of *in vivo* organelle markers for co-localization studies in *Arabidopsis* and other plants. Plant J 51: 1126-1136

Osakabe Y, Maruyama K, Seki M, Satou M, Shinozaki K, Yamaguchi-Shinozaki K (2005) Leucine-rich repeat receptor-like kinase1 is a key membrane-bound regulator of abscisic acid early signaling in *Arabidopsis*. Plant Cell 17: 1105-1119

Robert-Seilaniantz A, Grant M, Jones JD (2011) Hormone crosstalk in plant disease and defense: more than just jasmonate-salicylate antagonism. Annu Rev Phytopathol 49: 317-343

Schallus T, Jaeckh C, Fehér K, Palma AS, Liu Y, Simpson JC, Mackeen M, Stier G, Gibson TJ, Feizi T, et al (2008) Malectin: a novel carbohydrate-binding protein of the endoplasmic reticulum and a candidate player in the early steps of protein N-glycosylation. Mol Biol Cell 19: 3404-3414

Schweighofer A, Hirt H, Meskiene I (2004) Plant PP2C phosphatases: emerging functions in stress signaling. Trends Plant Sci 9: 236-243.

Smith LM, Bomblies K, Weigel D (2011) Complex evolutionary events at a tandem cluster of *Arabidopsis thaliana* genes resulting in a single-locus genetic incompatibility. PLoS Genet 7: e1002164
Stracke S, Kistner C, Yoshida S, Mulder L, Sato S, Kaneko T, Tabata S, Sandal N, Stougaard J, Szczegliowski K, et al (2002) A plant receptor-like kinase required for both bacterial and fungal symbiosis. Nature 417: 959-962

Tan X, Calderon-Villalobos Li, Sharon M, Zheng C, Robinson CV, Estelle M, Zheng N (2007) Mechanism of auxin perception by the TIR1 ubiquitin ligase. Nature 446: 640-645

Tanaka H, Osakabe Y, Katsura S, Mizuno S, Maruyama K, Kusakabe K, Mizoi J, Shinozaki K, Yamaguchi-Shinozaki K (2012) Abiotic stress-inducible receptor-like kinases negatively control ABA signaling in Arabidopsis. Plant J 70: 599-613

Ton J, Flors V, Mauch-Mani B (2009) The multifaceted role of ABA in disease resistance. Trends Plant Sci 14: 310-317

Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow TY, Hsing YI, Kitano H, Yamaguchi I, et al (2005) GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. Nature 437: 693-698

Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2000) Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. Proc Natl Acad Sci USA 97: 11632-11637

von Saint Paul V, Zhang W, Kanawati B, Geist B, Faus-Kessler T, Schmitt-Kopplin P, Schäffner AR (2011) The Arabidopsis glucosyltransferase UGT76B1 conjugates isoleucic acid and modulates plant defense and senescence. Plant Cell 23: 4124-4145

Walker-Simmons MK, Rose PA, Hogge LR, Abrams SR (2000) Abscisic acid. ABA immunoassay and gas chromatography/mass spectrometry verification. Methods Mol Biol 141: 33-47

Wang Y, Hua J (2009) A moderate decrease in temperature induces COR15a expression through the CBF signaling cascade and enhances freezing tolerance. Plant J 60: 340-349

Wells G, Huston C (1995) High-resolution selected ion monitoring in a quadrupole ion trap mass spectrometer. Anal Chem 67: 3650-3655
Wiermer M, Feys BJ, Parker JE (2005) Plant immunity: The EDS1 regulatory node. Curr Opin Plant Biol 8:383-389

Xin Z, Wang A, Yang G, Gao P, Zheng ZL (2009) The Arabidopsis A4 subfamily of lectin receptor kinases negatively regulates abscisic acid response in seed germination. Plant Physiol 149: 434-444

Yang SD, Seo PJ, Yoon HK, Park CM (2011) The Arabidopsis NAC transcription factor VNI2 integrates abscisic acid signals into leaf senescence via the COR/RD genes. Plant Cell 23: 2155-2168

Yu F, Qian L, Nibau C, Duan Q, Kita D, Levasseur K, Li X, Lu C, Li H, Hou C, et al (2012) FERONIA receptor kinase pathway suppresses abscisic acid signaling in Arabidopsis by activating ABI2 phosphatase. Proc Natl Acad Sci USA 109: 14693-14698
FIGURE LEGENDS

Figure 1. The *ios1-1* mutation impairs susceptibility to filamentous (hemi-)biotrophs. A, Transcriptional activation of *IOS1* by *Hpa*, as analyzed by transmission light microscopy for GUS activity (blue), 4 hours post inoculation (hpi) of the previously described *IOS1* reporter line (Hok et al., 2011). Non germinated spores (Sp) and spores penetrating from an appressorium (arrow) are visible attached to cotyledon surfaces (top). GUS staining is localized to the mesophyll cells underlying the penetration site (bottom, with a different optic focus). B, Transcriptional activation of *IOS1* by *Hpa*, as analyzed by dark-field microscopy of thin sections of cotyledons from the reporter line, 3 days post inoculation (dpi). GUS activity is shown in red. Cross sections (left) and tangential sections (right) reveal localized GUS activity in single-cell layers around invading hyphae (Hy) harboring haustoria (asterisks). C, Transcriptional activation of *IOS1* by *Phytophthora parasitica* (*Pp*), as shown by transmission light micrographs of *IOS1* reporter activity in root tips. In the absence of infection, *IOS1* is expressed in elongation zones only (left; compare with Fig. 3). Inoculation with *Pp* zoosporues activates the *IOS1* promoter during the initial biotrophic phase, in which hyphae invade cells from the root cap and the differentiation zone, 6 hpi (right). D, *Pp* disease is delayed in the *ios1-1* mutant. The photograph shows representative Wt (left) and mutant (right) plants, 18 dpi. The development of disease in Wt and *ios1-1* plants was assessed on the basis of an established score (Attard et al., 2010). The means and 95% two-tailed confidence intervals are shown. Differences in disease development between Wt and *ios1-1* plants were statistically significant (asterisk), as demonstrated by Scheirer-Ray-Hare nonparametric two-way analysis of variance (ANOVA) for ranked data (H=9.68; df=1; p=0.00186).

Figure 2. Impaired *ios1-1* susceptibility unlikely results from upregulated defense. A, Expression profile of genes coding for immune signaling elements in Arabidopsis. RT-qPCR analyses were performed with seedlings from *Hpa*-infected Wt and *ios1-1* mutant plants over a time course of 5 dpi. The data shown are the means and standard deviations of 3 biological replicates. B, Summary of the *p*-values associated to statistical analysis of the data presented in (A). For each gene, two factors (genotype and time) were treated as fixed (Model I of three-way ANOVA) and crossed. For each of the 30 tested individual situations (2 genotypes x 5 time points x 3 biological replicates), the mean expression of a technical triplicate was taken into account assuming a Gaussian distribution and the homoscedasticity. Analysis was performed using the software R.
RT-qPCR analysis for the expression of genes coding for PR proteins and a plant defensin in non-
inoculated and *Hpa*-infected plants from the Wt and the *ios1-1* mutant. Neither SA-, nor
JA/ethylene-mediated defense responses were upregulated in the *ios1-1* mutant. The
data shown are the means and standard deviations of 2 independent biological
replicates.

**Figure 3.** Tissue-specificity of *IOS1* expression in Arabidopsis organs at different
developmental stages. The transcriptional activation of *IOS1 in vivo* is revealed either by
enzymatic GUS activity (blue), or by GFP fluorescence in transgenic lines carrying the
*pIOS1::GFP-GUS* reporter gene construct. A-H, *IOS1* expression during vegetative
growth. I-O, *IOS1* expression during reproduction. Reporter activity is not detectable in
the embryo (A), but can be seen in the radicle emerging from the testa (B), indicating
transcriptional *IOS1* activation in the elongation zone (arrow). In primary roots, GUS
activity is limited to the elongation zone (C), and to root cap border cells undergoing
detachment (arrow). The *GUS* reporter gene is not transcribed in fully expanded root
cells (D and E), but the transcription of this gene is reinitiated in emerging (D) and
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In cotyledons and leaves, *IOS1* expression is restricted to the cells surrounding the
stomata, as shown by confocal microscopy of the GFP reporter (H). GFP appears in
green. Red spots represent chloroplasts within stomatal guard cells. GUS activity is
observed after pollination, in the style (l; arrow), corresponding to the development of
pollen tubes (K). During the subsequent development of the pollinated flowers, *IOS1*
activation occurs in the abscission zones of sepals and petals (L and M; arrows), in the
transmitting tract of developing fruits (N), and, finally, in the abscission zones of mature
siliques (O; arrow). Scale bars represent 10 µm (H), 50 µm (F and G), 100 µm (A-E, K
and L) and 500 µm (I,M,N, and O).

**Figure 4.** The *ios1-1* mutant is ABA-hypersensitive. A, Seed germination on medium
containing ABA is stronger inhibited in *ios1-1* seeds than in both the Wt and the
complemented mutant line *ios1-1cp4* (Hok et al., 2011). We show means and standard
deviations for radicle emergence rates determined for 3 plates, each containing about
100 seeds, for line and (±)-ABA concentration. B, The *ios1-1* mutant is more sensitive to
the ABA-induced inhibition of primary root elongation. Seven-day-old plantlets were
transferred to medium containing or not 10 µM (±)-ABA, and primary root elongation was measured 2, 4, 8, 9, and 10 days later. Shown are the means and standard errors of 30 individual plants/line. Without ABA, Wt, ios1-1, and complemented mutant plants attained similar mean primary root lengths of 73.4 (± 2.1), 73.1 (± 2.0), and 71.4 (± 1.5) mm, respectively, 10 days after transfer. C, Leaf surface temperature is higher in the ios1-1 mutant than in the Wt. Elevated leaf temperatures of ios1-1 were similar to those of the abh1 mutant (background Col). Shown are thermographs of a representative plant from each line, and the means and standard deviations of 20 measured replicates of 8 plants/line, which were obtained 48 h after exposure of the plants to continuous light. The significance of differences was determined in Student's t-test (identical letters, not significantly different with p>0.01; different letters, p<0.01). D, Stomatal development is not affected in the ios1-1 mutant. The micrographs illustrate representative, fully developed stomata on epidermal strips from rosette leaves of Wt and mutant plants. The graph shows the mean stomatal density and standard deviations of 4 independent experiments. E, The ios1-1 mutant is hypersensitive to the ABA-regulated inhibition of stomatal opening, but its ion channel-mediated stomatal movement appears normal. Plants were treated as indicated in the sketch above the graph. We show the mean aperture changes and standard deviations of 70 stomata on treated strips, based on the apertures of the same number of stomata from untreated controls from the mutant and Wt plants. F, Stomatal conductance (gH2O) in response to various concentrations of ABA (in µM). Values indicate the means and standard deviations of measurements of at least 5 plants per line and per treatment. Specific treatments of leaves prior to measurements are indicated in Materials and Methods. Asterisks in (A, B, and E) indicate significant differences, with p<0.01 as determined in Student's t-test. Asterisks in (F) indicate significant differences, with p<0.05, as determined by a modified Welch-Satterthwaite test implemented in the VANTED software package.

Figure 5. ABA signaling interfering with disease is attenuated by IOS1. A, RT-qPCR experiments to determine the time-course of ABA-regulated COR15A/B and RD29A/B marker gene transcript levels in Hpa-infected and non-infected Wt and ios1-1 plants. Represented are means and standard deviations of normalized relative quantities (NRQ), which were obtained for samples from 3 independent biological replicates. The insets represent the mean NRQ ratios and standard deviations between inoculated and non-inoculated plants from the same genotype at 8 and 24 hpi. The table below the graphs summarizes the p-values that were associated to statistical analysis of the data. For
each gene, three factors (genotype, treatment and time) were treated as fixed (Model I of three-way ANOVA) and crossed. For each of the 60 tested individual situations (2 genotypes x 2 treatments x 5 time points x 3 biological replicates), the mean expression of a technical triplicate was taken into account. Analysis was performed using the software R. *, 0.05>P>0.01; **, 0.01>P>0.001; ***, P<0.001. B, Representative cotyledons from the Wt (left panels) and the ios1-1 mutant (right panels) harboring the pRD29B::GUS construct, 3 days after control treatment (water), or post inoculation with Hpa. ABA signaling and reporter gene expression were either not particularly induced (Untreated), or stimulated by spraying ABA onto cotyledons (ABA), or by lowering relative humidity (Drought). For a compiled view of the experiment, and for quantitative analyses, see Supplemental Fig. S7. C, Exogenous application of ABA (10 µM) decreases the reproduction of Hpa on the Wt, and the complemented ios1-1 mutant (ios1-1cp4). It does not further decrease the lowered susceptibility of ios1-1. D, ABA signaling interferes with Hpa infection. The Ler wild-type and mutants in this background (abi1-1, ios1-1) were inoculated with the compatible Hpa isolate Wela. The Col wild-type and the abh1 mutant were inoculated with the compatible isolate Noco2. The values shown in C and D are means and standard errors of 20 replicates. The significance of differences was determined in Student’s t-test (different letters, p<0.001). FW, Fresh weight.
SUPPLEMENTAL DATA

Supplemental Figure S1. IOS1 affects powdery mildew disease, but not bacterial wilt.

A, Transcriptional activation of IOS1 upon infection by the fungal biotroph, Erysiphe cruciferarum, at 2 and 4 dpi. Fungal running hyphae, stained with 10% blue ink (v/v) in 25% acetic acid (v/v) (Hückelhoven and Kogel, 1998), penetrate epidermal cells and activate IOS1 promoter-driven GUS expression in mesophyll cells. The arrows indicate conidia from which the fungus developed. B, Powdery mildew disease symptoms on the leaves of 4-week-old Arabidopsis, 10 dpi. We inoculated five plants from each line at the rosette stage. The most severely affected leaves from each plant are shown. Mildew proliferation levels were lower on ios1-1 leaves than on the Wt. C, Sporulation of powdery mildew from microcolonies (n=125 on 15 leaves pooled from 3 independent inoculations) on the leaves of four-week-old Arabidopsis, 5 dpi. The means and 95% two-tailed confidence intervals are shown, and the difference between the means was significant, according to Student’s t-test (*, p<0.05). D, Development of bacterial wilt on Wt plants and the ios1-1 mutant. The roots of four-week-old plants were inoculated with the virulent R. solanacearum strain GMI1000. Disease symptoms on inoculated plants were scored 2, 5, 6, 7, and 8 days post inoculation, according to a published disease index (Hernández-Blanco et al., 2007). The results shown are the mean scores and standard deviations of 20 inoculated plants.

Supplemental Figure S2. ios1-1 mutants have no aberrant developmental phenotypes.
Arabidopsis was grown in vitro and plants were photographed at 12 and 28 days after sowing. Plants were then transferred to soil, and pictures were taken 33 and 60 days after sowing. Flowering and seed set were not affected by the mutation.

Supplemental Figure S3. The ios1-1 mutation does not affect callose deposition in powdery mildew-infected leaves. Shown are 3 infected areas of Wt and ios1-1 leaves at 5 dpi. Callose (blue) and fungal structures (red) were stained with methyl blue and wheat germ agglutinine-tetramethylrhodamin (WGA-TMR), respectively. Smaller callose deposits (arrowheads) highlight penetration sites and encased haustoria. Bigger callose deposits (arrows) represent partially encapsulated cells. Leaves were cleared in ethanol:acetic acid (6:1, v/v) overnight, washed with water, incubated overnight in 0.05% methyl blue (w/v 0.067 M K-phosphate buffer at pH 9.2), and washed in water. Fungal structures were visualized by incubation of the leaves for 20 min in PBS buffer (pH 7.4)
and transfer to WGA-TMR-staining solution (10 µg mL⁻¹ WGA-TMR in PBS with 10 µg mL⁻¹ BSA). After vacuum infiltration, leaves were left in the staining solution at least over night at 4°C in the dark. WGA-TMR was exited by a 561 nm laser line and the emission detected at 571-610 nm. Methyl blue was excited at 458 nm and the detection was set to the wavelengths from 470-490 nm (upright confocal microscope Leica SP5, Leica, Wetzlar Germany). Bars, 100 µm.

**Supplemental Figure S4.** Abscisic acid (ABA) does not induce IOS1 expression. A, Treatment of leaves from the prIOS1::GFP-GUS reporter line with ABA did not result in detectable GUS activity. We show a representative leaf, 3 days after the infiltration of 10 µM ABA. B and C, IOS1 is expressed in the absence of ABA. ABA was eliminated from 2-week-old in vitro-grown prIOS1::GFP-GUS reporter plants by supplementing the medium with 100 µM 1-methyl-3-phenyl-5-[3-(trifluormethyl)phenyl]-4-pyridinone (fluridon, an inhibitor of phytoene desaturase in carotenoid biosynthesis) for 3 days. Primary (B) and secondary (C) roots showed severe elongation defects, but still expressed IOS1.

**Supplemental Figure S5.** ABA levels in cotyledons from Arabidopsis plants. Samples from the Wt, the ios1-1 mutant, and the complemented line ios1-1cp4 were collected before (time point 0), and 2 and 4 days after spray-inoculation with Hpa, freeze-dried and submitted to analysis as indicated in Materials and Methods. ABA levels were low in cotyledons, and no significant differences between genotypes were observed according to the Kruskal-Wallis non-parametric H-test (p<0.05). Shown are the means (±SD) from three biological replicates, each composed of two technical replicates.

**Supplemental Figure S6.** The ABA-induced transient expression of COR genes is accelerated in the ios1-1 mutant. Cotyledons of 8 day-old seedlings were sprayed to saturation with water or 5 µM ABA, and kept in a water-saturated atmosphere until sample collection 1 h, 2 h, 4 h, and 6h after onset of treatment. Total RNA extracted from these samples was submitted to RT-qPCR with gene-specific primers for COR15A and COR15B. Samples from cotyledons that were sprayed with a lower ABA concentration (0.5 µM; insets) did not reveal stimulated COR gene expression, when compared to water-treated controls, and were analyzed only at 1h and 2 h after onset of treatment. Represented are the means and standard deviations of normalized relative quantities that were obtained for samples from 3 independent biological replicates.
Supplemental Figure S7. ABA-sensitive reporter gene expression is downregulated in 
Hpa-inoculated Wt, but not in ios1-1. A, Overview of 15 cotyledons per treatment from 
seedlings that harbor the pRD29B::GUS reporter construct in the Wt and ios1-1 
background. Seedlings were spray-inoculated with water or Hpa prior to applying ABA or 
the drought stress, and stained with X-Gluc at 3 dpi. In the Wt, GUS activity was 
increased by ABA application and drought stress, but reduced in all situations upon Hpa 
inoculation. GUS activity was maintained in cotyledons from the ios1-1 mutant upon 
infection. B, Fluorimetric quantification of the pathogen-responsive, pRD29B-dependent 
GUS activity in cotyledons from the Wt and the ios1-1 mutant. Cotyledons from about 
100 plants per line and treatment were pooled, and the extracted proteins were 
submitted to enzymatic GUS assays with MUG as the substrate (Jefferson et al., 1987). 
The data shown are the means and standard deviations of 3 independent biological 
replicates, each performed with 3 technical replicates. Asterisks indicate significant 
differences (p<0.001) as determined with Student’s t-test.

Supplemental Figure S8. Proposed role of IOS1 during infection. (Hemi-)biotrophic 
filamentous pathogens induce IOS1 expression to downregulate ABA signaling during 
the establishment of disease in Arabidopsis. In the absence of IOS1, the downregulation 
of ABA signaling is compromised in ios1-1, impairing the infection process. The 
micrograph shows a secondary infection by Phytophthora parasitica of a leaf from the 
pIOS1::GFP-GUS reporter line. Hyphae (Hy) on the leaf surface re-enter through 
stomata (arrow) and activate IOS1 expression (blue) in the underlying mesophyll cells.

Supplemental Figure S9. Transcripts produced by ios1 mutants. A, The graph 
represents insertion sites (triangles) for ios1-1 in a region encoding the malectin-like 
domain, and for ios1-2 and ios1-3 in a region encoding the kinase domain. Grey boxes 
indicate exons, small red boxes indicate UTRs. The arrows and numbers below indicate 
primers (see Table S1) and their attachment sites. B, RT-PCR with the indicated primers 
and RNA extracted from the mutants. Primer pairs were chosen to detect amplicons 
upstream the insertion, spanning the insertion, and downstream the insertion. Amplicons 
upstream the insertions indicate the formation of truncated mRNAs by all 3 allelic 
mutants. Only ios1-3 forms also a truncated transcript 3' of the insertion location. This 
possibly indicates a transcription start site in the inserted T-DNA, which previously has 
been reported for other insertion mutants (e.g. bak1-3; Chinchilla et al., 2007). NADH, 
constitutive control gene encoding a NADH-ubiquinone oxidoreductase 20 kDa subunit
on locus At5g11770. C, Interpretation of the obtained data. The allelic mutants *ios1-2*
and *ios1-3* produce transcripts, which are potentially translated into receptor-like
proteins. This might account for the absence of phenotypes related to ABA
hypersensitivity and the interaction with *Hpa*. MLD, malectin-like domain; LRR, leucine-
rich repeats; TM, membrane-spanning domain.

**Supplemental Table S1.** Accession numbers, names and primer sequences for genes
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Figure 3. Tissue-specificity of *IOS1* expression in Arabidopsis organs at different developmental stages. The transcriptional activation of *IOS1* in vivo is revealed either by enzymatic GUS activity (blue), or by GFP fluorescence in transgenic lines carrying the *pIOS1::GFP-GUS* reporter gene construct. A-H, *IOS1* expression during vegetative growth. I-O, *IOS1* expression during reproduction. Reporter activity is not detectable in the embryo (A), but can be seen in the radicle emerging from the testa (B), indicating transcriptional *IOS1* activation in the elongation zone (arrow). In primary roots, GUS activity is limited to the elongation zone (C), and to root cap border cells undergoing detachment (arrow). The GUS reporter gene is not transcribed in fully expanded root cells (D and E), but the transcription of this gene is reinitiated in emerging (D) and elongating (E) secondary roots. In hypocotyls, GUS activity is detected only during etiolation, in the dark (G), with no activity detected in non etiolated tissues in the light (F). In cotyledons and leaves, *IOS1* expression is restricted to the cells surrounding the stomata, as shown by confocal microscopy of the GFP reporter (H). GFP appears in green. Red spots represent chloroplasts within stomatal guard cells. GUS activity is observed after pollination, in the style (I; arrow), corresponding to the development of pollen tubes (K). During the subsequent development of the pollinated flowers, *IOS1* activation occurs in the abscission zones of sepals and petals (L and M; arrows), in the transmitting tract of developing fruits (N and O; arrows). Scale bars represent 10 μm (H), 50 μm (F and G), 100 μm (A-E, K and L) and 500 μm (I, M, N, and O).
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