Early Responses in the *Arabidopsis-Verticillium longisporum* Pathosystem Are Dependent on *NDR1*, JA- and ET-Associated Signals via Cytosolic NPR1 and *RFO1*

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The responses of *Arabidopsis* accessions and characterized genotypes were used to explore components in the early defense responses to the soilborne fungus *Verticillium longisporum*. *V. longisporum* susceptibility was found to be a complex trait, in which different disease phenotypes, such as stunting, altered flowering time, weight loss, and chlorosis were perceived differently across genotypes. A Bay-0 × Shahdara recombinant inbred line population was used to identify two loci on chromosomes 2 and 3 of Bay-0 origin that caused enhanced chlorosis after *V. longisporum* challenge. Furthermore, the observation that a mutation in *RFO1* in Col-0 resulted in susceptibility whereas the natural *rfo1* allele in Ty-0 showed a high degree of resistance to the pathogen supports the hypothesis that several resistance quantitative trait loci reside among *Arabidopsis* accessions. Analysis of mutants impaired in known pathogen response pathways revealed an enhanced susceptibility in *ein2-1*, *ein4-1*, *ein6-1*, *esa1-1*, and *pad1-1*, but not in other jasmonic acid (JA)-, ethylene (ET)-, or camalexin-deficient mutants, suggesting that *V. longisporum* resistance is regulated via a hitherto unknown JA- and ET-associated pathway. Pretreatments with the ET precursor 1-aminocyclopropane-1-carboxylic acid (ACC) or methyl jasmonate (MeJA) caused enhanced resistance to *V. longisporum*. Mutants in the salicylic acid (SA) pathway (*eds1-1*, *NahG*, *npr1-3*, *pad4-1*, and *sid2-1*) did not show enhanced susceptibility to *V. longisporum*. In contrast, the more severe *npr1-1* allele displayed enhanced *V. longisporum* susceptibility and decreased responses to ACC or MeJA pretreatments. This shows that cytosolic NPR1, in addition to SA responses, is required for JA- and ET-mediated *V. longisporum* resistance. Expression of the SA-dependent *PR-1* and *PR-2* and the ET-dependent *PR-4* were increased 7 days postinoculation with *V. longisporum*. This indicates increased levels of SA and ET in response to *V. longisporum* inoculation. The *R*-gene signaling mutant *ndr1-1* was found to be susceptible to *V. longisporum*, which could be complemented by ACC or MeJA pretreatments, in contrast to the *rfo1* T-DNA mutant, which remained susceptible, suggesting that *RFO1* (*Fusarium oxysporum* resistance) and *NDR1*

(nonrace specific disease resistance 1) activate two distinct signaling pathways for *V. longisporum* resistance.

Additional keywords: natural variation, QTL, *Verticillium* wilt.

Plants are under constant attack by a vast array of pathogens. In response to attack, plants express numerous defense mechanisms, either preformed or activated upon pathogen recognition (Hammond-Kosack and Parker 2003; Nimchuk et al. 2003). Studies of the molecular mechanisms underlying activation of the various defense responses over the last ten years have, however, enhanced our understanding of these exceedingly complex networks of interactions. The signal transduction pathways mediated by salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) are known to play central roles in the activation of plant defense responses (Glazebrook 2001, 2005). Recognition of a pathogen often triggers a localized resistance reaction known as the hypersensitive response (HR), which is characterized by rapid cell death at the site of infection (Hammond-Kosack and Jones 1996). A HR or attack by certain pathogens induces elevation of endogenous SA levels and triggers activation of SA-dependent signaling and expression of pathogenesis-related (*PR*) genes, like *PR-1*, *PR-2*, and *PR-5* (Gaffney et al. 1993; Linthorst 1991; Uknes et al. 1992). JA and ET, on the other hand, coregulate another subset of *PR* genes comprising *PR-3*, *PR-4*, and *PR-12*, which are not dependent on NPR1/NIM1 (nonexpressor of PR-1 and nonimmunity 1) (Fan and Dong 2002; Penninckx et al. 1998; Thomma et al. 1998). NPR1 has been identified as a key component of the SA signal transduction pathway (Cao et al. 1994; Delaney et al. 1994; Glazebrook et al. 1996; Shah et al. 1997), which acts as a modulator of *PR* gene expression but does not bind DNA directly (Després et al. 2000). However, some SA-dependent defense responses are independent of *NPR1* (Clarke et al. 2000; Uquillas et al. 2004) or involve feedback loops (Falk et al. 1999; Jirage et al. 1999). Further studies of NPR1 have revealed interactions of transcription factors that are required for the SA response and *PR* gene expression (Fan and Dong 2002; Johnson et al. 2003; Zhang et al. 2003). For the expression of *PR-12* (a plant defensin, also named *PDF1.2*) to take place, a concomitant activation of the JA and ET pathways are required (Mackerness et al. 2000; Penninckx et al. 1998). The SA, JA, and ET responses show considerable overlap following wounding (Arimura et al. 2002; Cheong et al. 2002; Moran and Thompson 2001). Furthermore, local responses to wound-

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ing can trigger systemic acquired responses (SAR), which confer long-lasting protection against a broad spectrum of microorganisms (Durrant and Dong 2004; Ross 1961). The onset of SAR is accompanied by a local and systemic increase in endogenous levels of SA and an induction of the *PR* genes (Delaney et al. 1994; Gaffney et al. 1993; Ryals et al. 1996; Sticher et al. 1997; van Loon and Van Strien 1999). Pathogen-induced SAR requires SA, in contrast to induced systemic resistance (ISR), which is activated upon colonization of roots by selected strains of nonpathogenic rhizobacteria (Pieterse et al. 2001, 2002). In the ISR signaling pathway, components from the JA and ET responses act in sequence in triggering a defense reaction that converges with SA signaling in NPR1 dependency (Pieterse et al. 1998, 2001; Ton et al. 2002). ISR is initiated in roots, whereas SAR is initiated in leaves, suggesting that these two responses may not be in competition for NPR1 (Iavicoli et al. 2003). When SA and JA are applied together to leaves, the presence of SA inhibits JA synthesis and signaling. This inhibition is alleviated in the *npr1* mutant, indicating that NPR1 is part of the cross-talk control between the two signaling pathways (Spoel et al. 2003). Dissection of the SA, JA, and ET signaling network via genome-wide transcription studies on *Arabidopsis* mutants impaired in those three pathways revealed extensive cross-talk (Glazebrook et al. 2003; Schenk et al. 2000). In a parallel transcript-profiling study, no genes systemically induced by *Pseudomonas fluorescens* were found, but a set of genes predicted to be regulated via JA and ET signaling were primed to respond faster or more strongly upon *P. syringae* pv. *tomato* attack after rhizobacteria challenge (Verhagen et al. 2004).

Verticillium longisporum is a soilborne fungal pathogen, which especially thrives in northern Europe where it particularly attacks *Brassica* oil crops (Fahleson et al. 2003; Johansson et al. 2006; Zeise and Tiedemann 2002). The infection process of *V. longisporum* and the closely related *V. dahliae* is very similar. It starts with the germination of microsclerotia, resting structures present in the soil, in response to host root exudates. The fungus enters the root and immature xylem elements, followed by multiple events including hyphal proliferation and the production of conidia. The conidia are spread upstream in the vascular elements. As the plant begins to senesce, the fungus leaves the xylem elements and colonizes the surrounding nonvascular tissues and microsclerotia formation is initiated. Not much is known about induced plant-defense responses to soilborne pathogens, compared with leaf pathogens. Whether soilborne pathogens resemble any signaling features similar to rhizobacteria or have necrotrophic signatures is far from elucidated. To expand our knowledge of defense responses to this pathogen, we evaluated its interaction with a wide range of *Arabidopsis* accessions and mutants impaired in well-characterized defense pathways. The study revealed that *V. longisporum* resistance and disease is governed by multiple quantitative trait loci (QTL) with complex interactions regarding development, aging, and flowering time. In contrast to rhizobacteria-induced responses and *V. dahliae* resistance, a requirement for ET- and JA-associated components was found. A cell wall-associated kinase-like gene involved in *Fusarium oxysporum* resistance (*RFO1*) together with the *R*-gene signaling component *NDR1* (nonrace-specific disease resistance 1) were discovered to be essential for *V. longisporum* resistance.

RESULTS AND DISCUSSION

Multiple resistance QTL to *V. longisporum* reside in *Arabidopsis* accessions.

To evaluate the natural variability of resistance against *V. longisporum*, 169 *Arabidopsis* accessions were monitored using

an in vitro-based screening procedure (Steventon et al. 2001). All plants were individually scored, based on the number of days until the opening of the first flower, plant height, and typical disease symptoms such as discoloration, chlorosis, and microsclerotia formation. A total of 54 accessions were scored to be resistant, being as unaffected as the water-inoculated control plants, whereas 115 accessions exhibited various degrees of enhanced susceptibility. Col-0, *Ler*-0, and *Ws*-0 were all slightly stunted when inoculated with *V. longisporum* (Fig. 1A and B). The accessions Cha-0 and Je-0 displayed the highest degree of susceptibility (Fig. 1C and D), being very stunted already at day 10. Shahdara was one of the most resistant accessions (Fig. 1E) in contrast to Bay-0 (Fig. 1F). In order to genetically map resistance loci, four parental accessions to recombinant inbred lines (RIL) were further assessed. A total of 5% of

Fig. 1. Responses of *Arabidopsis* genotypes 10 days after inoculation of water (to the left) and *Verticillium longisporum* (to the right). **A**, Col-0 and **B**, *Ler*-0 with intermediate response to the fungus. **C**, Cha-0 and **D**, Je-0 showing enhanced susceptibility. **E**, Shahdara showing high tolerance to *V. longisporum* and **F**, Bay-0 with intermediate response to *V. longisporum*. **G**, The T-DNA mutant salk_077975 in *RFO1* with enhanced susceptibility to *V. longisporum* compared with Col-0 background and **H**, the natural *RFO1* mutant in Ty-0 (*rfo1*_{Ty-0}) showing tolerance to *V. longisporum*.

Col-0 plants and 15% of *Ler*-0 plants had at least one chlorotic leaf 10 days after inoculation, whereas this phenotypic response was found in 20% of Shahdara plants and as many as 40% of the Bay-0 plants. Transgressive segregation was only observed in the Bay-0 × Shahdara RI lines (Loudet et al. 2002), in which formation of chlorosis ranged from 0 to 55% 10 days after inoculation. The latter group showed a clear wilting phenotype of the entire plant. Composite interval mapping was used to identify susceptibility loci. The markers MSAT 2.27 inherited from Bay-0 on chromosome 2 (log of the likelihood ratio (LOD) = 4.42) and MSAT 3.21 inherited from Bay-0 on chromosome 3 (LOD = 3.5) were both associated with enhanced susceptibility. An additive epistasis was seen between MSAT 2.27 and MSAT 3.21 that corresponded to a significant effect on chlorosis formation ($P = 0.006$). Despite stronger penetrance of the chlorosis trait in some of the Bay-0 × Shahdara RIL as compared with Bay-0, no chlorosis-associated markers of Shahdara origin could be identified.

Furthermore, to evaluate if wall-associated kinase-like 22 (WAKL22/*RFO1*), a gene recently found to regulate resistance to *Fusarium oxysporum*, another fungus causing vascular wilt disease (Diener and Ausubel 2005), also had an impact on defense to *V. longisporum*, the salk insertion line 077975, which contains a T-DNA that disrupts the first exon of At1g79670, was inoculated. The *rfo1* T-DNA insertion mutant was clearly more susceptible, exhibiting a fresh weight (FW) loss of 87%, compared with wild-type Col-0 with a FW loss of 65% (Fig. 1G). The salk 140705 mutant inserted 615 bp upstream of the ATG translational start codon of At1g79670 showed a less dra-

matic response (73% FW loss). In contrast, the Ty-0 accession with the natural *rfo1* allele showed a relatively high degree of resistance to *V. longisporum* (Fig. 1H), indicating compensation from other resistance loci in Ty-0. Any differentiated responses between Col-0 and C24, as reported from the genetic mapping of the *V. dahliae* locus (*VET1*) on chromosome 4 (Veronese et al. 2003), were not observed in the present study. The genetic data collected so far on resistance genes to *Verticillium* spp. present in *Arabidopsis* emphasizes the genetic complexity when various loci reside in different accessions. These loci may also play important roles in conferring resistance at various developmental stages.

***V. longisporum* resistance in *Arabidopsis* requires JA- and ET-associated responses via cytosolic NPR1.**

To assess the role of SA, JA, and ET signaling in defense against *V. longisporum*, *Arabidopsis* mutants impaired in those pathways were inoculated. The plant responses were recorded by comparing induced changes in FW (control vs. inoculated), to obtain an overall disease-severity grading of this wilt-inducing fungus. Of the ET-insensitive mutants (*ein2-1*, *ein3-1*, *ein4-1*, *ein6-1*, *etr1-1*) and the ET-overproducing mutant (*etol-1*) screened, only the ET receptor *ein4-1*, together with *ein2-1* and *ein6-1*, two downstream components in the ET pathway, showed enhanced susceptibility. A hypersusceptible response was found in *ein6-1*, and all the plants were dead already five days after *V. longisporum* inoculation, whereas *ein2-1* and *ein4-1* exhibited enhanced susceptibility 10 days after inoculation (Table 1, Fig. 2A). *ein3-1* and *etol-1* had similar responses to Col-0 after fungal inoculation, and *etr1-1* was even more tolerant than wild type (Fig. 2B). This indicates that prolonged ET perception via *ETR1* but not *EIN4* enhances susceptibility to *V. longisporum* via induction of senescence, as seen in previous studies after *V. dahliae* challenge (Boller 1991; Robinson et al. 2001; Tjamos et al. 2005; Veronese et al. 2003). *EIN6* is involved in both ET signaling and mechanical stimulation including wind, touching, and rubbing (Wright et al. 2002). However, in our case the uninoculated *ein6-1* control plants did not show any similarities to the hypersusceptible phenotype, which shows that the response observed is caused by *V. longisporum*, linked to ET signaling and not to any factor induced in the culture regime used. The stronger phenotype seen on the *ein6-1* mutant compared with *ein4-1* and *ein2-1* implies that additional signals activate *EIN6*-dependent resistance. Interestingly, two mutants affecting induction of the phytoalexin camalexin, JA, and reactive oxygen intermediates (ROI) signaling, *pad1-1* and *esal-1*, were both clearly susceptible (Fig. 2C). However, *pad3-1*, in which camalexin production is completely abolished, did not show any changed response, which shows that camalexin is not a key component in this defense system. In addition, none of the *jar1-1*, *coil-16*, and *eds8-1* mutants impaired in JA signaling showed any enhancement of susceptibility as compared with wild-type in response to *V. longisporum*, which suggests an independency of JA signaling (Fig. 2D). This observation is in contrast to enhanced susceptibility on *jar1-1* recently reported from *V. dahliae* challenge (Tjamos et al. 2005) and the role of JA in tomato (Thaler et al. 2004).

A clear difference between the mutant alleles *npr1-1* and *npr1-3* could furthermore be seen, in which only *npr1-1* showed a reduction in FW compared with Col-0 after inoculation with *V. longisporum* (Table 1, Fig. 2E). From microarray analysis of responses to *Pseudomonas syringae* pv. *maculicola* (Glazebrook et al. 2003), a combined effect of JA and ET signaling was observed in *pad1-1*, but the mutant was conferred to the JA pathway. The transcriptional analysis also showed that the expression of SA-mediated genes and genes whose expression requires JA and ET signaling was affected in the

Table 1. Changes in fresh weight of *Arabidopsis* defense-signaling mutants upon challenge with *Verticillium longisporum* 10 days postinoculation as compared with uninoculated plants

<i>Arabidopsis</i> genotype	Relative value ^a	Implicated in ^b
Col-0	100	
<i>npr1-1</i>	53	SA/ET/JA signaling
<i>npr1-3</i>	94	SA signaling
<i>eds1-1^c</i>	135	SA/R gene signaling
<i>pad4-1</i>	138	SA/R gene signaling
<i>sid2-1</i>	123	SA signaling
<i>NahG</i>	109	SA signaling
<i>ein2-1</i>	65	ET signaling
<i>ein3-1</i>	106	ET signaling
<i>ein4-1</i>	76	ET signaling
<i>ein6-1^c</i>	Nd ^d	ET signaling
<i>etol-1</i>	91	ET signaling
<i>etr1-1</i>	138	ET signaling
<i>eir1-1</i>	91	ET signaling
<i>eds8-1</i>	126	JA signaling
<i>jar1-1</i>	118	JA signaling
<i>coil-16</i>	82	JA signaling
<i>pad1-1</i>	53	JA/camalexin
<i>pad3-1</i>	91	camalexin
<i>esal-1</i>	68	JA/ROS/camalexin
<i>abi1-1^d</i>	125	ABA signaling
<i>abi4-1</i>	103	ABA signaling
<i>aba1-3^e</i>	87	ABA signaling
<i>aba2-1</i>	68	ABA signaling
<i>ndr1-1</i>	65	R gene signaling
<i>rar1-13^e</i>	122	R gene signaling

^a Relative fresh weight was measured as the relationship of lost fresh weight between inoculated plants and uninoculated plants 10 days postinoculation (dpi). Each observation is based on 24 plants, and the experiment has been repeated at least three times. The data has been normalized towards Col-0 and remaining weight percentage (100 = 34%).

^b SA = salicylic acid, ET = ethylene, JA = jasmonic acid, ROS = reactive oxygen species, and ABA = abscisic acid.

^c Ws background to which the data is normalized (100 = 28%).

^d Not determined. Inoculated plants dead at 5 dpi.

^e *Ler* background to which the data is normalized (100 = 32%).

Fig. 2. A selection of resistant and susceptible responses of *Arabidopsis* mutants inoculated with *Verticillium longisporum* isolate VD11 10 days postinoculation. Plants inoculated with water to the left and fungal inoculation to the right. **A**, An enhanced susceptibility was found on the ethylene (ET) receptor mutant *ein4-1*, whereas **B**, the ET receptor mutant *etr1-1* is slightly more resistant than wild type. **C**, The mutant *pad1-1*, impaired in jasmonic acid (JA)-associated responses, showed an enhanced susceptibility, but **D**, the JA-resistant mutant *jar1-1* had a response indistinguishable from wild type. **E**, The *npr1-1* mutant displayed an enhanced susceptibility, but **F**, salicylic acid-deficient *sid2-1* displayed an enhanced resistance. **G**, The *ndr1-1* mutant displayed an enhanced susceptibility, which could be complemented **H**, by methyl jasmonate pretreatment. **I**, The *aba2-1* mutant displayed an enhanced susceptibility.

npr1-1 mutant, which destabilizes NPR1 due to a substitution of histidine 334 to tyrosine. However, in the *npr1-3* mutant, which lacks the carboxyterminal 194 amino acids (truncated after residue 399) that contain the nuclear-localization signal, only SA-mediated gene expression was compromised. This is consistent with the observation that the truncated NPR1 is cytoplasmically localized in *npr1-3* and still is partially active in cross-talk (Dong 2004; Pieterse and van Loon 2004). None of the additional four genotypes impaired in SA signaling (*NahG*, *sid2-1*, *eds1-1*, *pad4-1*) exhibited enhanced susceptibility to *V. longisporum* as compared with wild type (Table 1). The mutants *sid2-1* (Fig. 2F) and *pad4-1* were even found to be less affected, compared with wild-type Col-0. Taken together, it cannot be ruled out that SA, JA, and ET all influence resistance to *V. longisporum*. Since *ein2-1*, *ein4-1*, *ein6-1*, *esal*, *pad1-1*, and *npr1-1* cause the most obvious susceptible responses, ET and, partially, JA seem to play more important roles than SA. It can be hypothesized, based on the differential responses observed, that ET and JA signaling in this pathosystem is regulated via hitherto unknown components and that the enhanced resistance in some SA mutants is due to an antagonistic response by SA. The results reported on the activity of the *Paenibacillus alvei* K165 strain does, however, show that the biocontrol-induced response against *V. dahliae* in *Arabidopsis* is dependent of SA via *NPR1* (Tjamos et al. 2005), which further suggests that SA also has a positive influence on resistance at a later stage of development and under different growth conditions.

ET is produced post-*V. longisporum* inoculation, independently of root ET sensitivity.

Earlier studies of exogenous ET pretreatment have shown aggravated disease symptoms of tomato to *V. albo-atrum* (Cronshaw and Pegg 1976) and enhanced ET production in cotton upon *V. dahliae* attack (Boller 1991). To elucidate possible roles of ET production in our system, *eir1-1*, a mutant insensitive to ET in roots only, and a *V. longisporum* disease phenotype indistinguishable from Col-0 were inoculated, and ET measurements undertaken. No difference in ET production was found at 1 and 3 days postinoculation (dpi) compared with wild-type. At 5 dpi, inoculated Col-0 plants produced approximately fivefold ET compared with control plants (3.9 versus 0.8 ppm), while inoculated *eir1-1* plants produced almost sevenfold ET compared with control plants (Fig. 3). At this timepoint also, visible disease symptoms including chlorosis

Fig. 3. Measurement of total ethylene (ET) production by Col-0 and *eir1-1* plants mock-treated or inoculated with *Verticillium longisporum* (VI). Mean values and standard deviations are based on 10 to 20 samples. The experiment was repeated twice.

were present on both genotypes. At 8 dpi, the accumulated ET had increased in both Col-0 and *etr1-1* samples to 6.6 and 7.9 ppm, respectively. The results show that *V. longisporum* induces ET production and that this reaction, in contrast to nonpathogenic rhizobacteria (Knoester et al. 1999), is independent of the ET insensitivity of the roots, hence demonstrating that *V. longisporum* responses and ISR trigger two different defense mechanisms.

Induced responses

to *V. longisporum* after chemical pretreatments.

To further clarify and confirm the role of SA, JA, and ET signaling observed in response to *V. longisporum*, seven genotypes, *etr1-1*, *jar1-1*, *npr1-1*, *npr1-3*, *sid2-1*, *NahG*, together with Col-0, were pretreated with 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ET, methyl jasmonate (MeJA), or SA three days before fungal inoculation. None of the pretreatments compromised fungal growth in the media (data not shown). The effect of *V. longisporum* inoculation on FW was evaluated in relation to uninoculated control plants under identical pretreatment conditions. Mock-treated Col-0 plants retained 30% of FW compared with the uninoculated controls. ACC and MeJA pretreatment significantly ($P < 0.05$) increased FW of the inoculated plants to approximately 50 and 75% of the uninoculated control plants, respectively. Pretreatment with SA did not show any significant alterations in FW loss compared with mock-treated material (Fig. 4). The *etr1-1* mutant did not show a FW increase after ACC treatment, compared with mock-treated material, whereas MeJA pretreatment still significantly ($P < 0.05$) reduced the effect from inoculation to a level comparable to the wild-type. The effect of ACC and MeJA treatment on *jar1-1* was less than the effect seen on Col-0. MeJA pretreatment did, however, still significantly ($P < 0.05$) decrease the FW loss compared with *jar1-1* without pretreatment. The responses in *etr1-1* and *jar1-1* to ACC and MeJA pretreatments suggest that JA acts downstream of ET in this defense response. In *Arabidopsis*, several necrotrophs are hampered in their containment as a response to JA (Thaler et al. 2004). The same authors found, on the other hand, that a JA treatment of the tomato *def1* mutant resulted in an enhanced

susceptibility to *V. dahliae*, which highlights the question of differential host responses, timing of the response, various cross talk, and generalizations between systems.

SA pretreatment increased the FW loss from *V. longisporum* inoculation in the *etr1*, *jar1-1*, *NahG*, and *sid2* genotypes compared with mock-treated controls, indicating that SA-dependent inhibition of JA enhances susceptibility to *V. longisporum*. Further confirmation of this mechanism can be found in the *npr1-1* and *npr1-3* mutants, which are impaired in the negative cross-talk between SA and JA (Spoel et al. 2003). SA treatments of the two *npr1* mutants show an enhanced resistance to *V. longisporum* but were less responsive to ACC and MeJA pretreatment compared with Col-0. This indicates that ET- and JA-induced resistance to *V. longisporum* requires *NPRI*, which is similar to what has been observed for JA- and ET-dependent resistance via *NPRI* in ISR (Pieterse et al. 1998). The reduced responsiveness to ACC and MeJA pretreatments in *NahG* are more confusing but may be caused by a higher endogenous JA response in these plants or, alternatively, that a basal level of SA is required for modulation or potentiation of ET- and JA-induced resistance via an increase of *NPRI* transcripts (van Wees et al. 2000). A potentiation of *NPRI*-mediated JA responses via low levels of SA has recently been observed in tobacco and *Arabidopsis* (Mur et al. 2006). The SA biosynthesis mutant *sid2-1* does, however, respond like wild type to ACC and MeJA treatments, which indicates that the effect seen in *NahG* plants may be due to pleiotropic effects (Van Wees and Glazebrook 2003).

Arabidopsis-*V. longisporum* resistance is independent of EDS1 but requires NDR1.

Information obtained about defense signaling thus far shows that the coiled coil-nucleotide binding-leucine-rich repeat (CC-NB-LRR) and toll and interleukin-1 receptor (TIR)-NB-LRR R proteins have differential requirements for downstream signaling components. Many CC-NB-LRR class R proteins require the function of NDR1, which is a plasma membrane localized protein (Coppinger et al. 2004) that structurally belongs to the HIN1 (harpin-induced 1) protein family but are independent of EDS1 (enhanced disease susceptibility). The lipase-like proteins EDS1 and PAD4 (phytoalexin deficient 4) are, however, required by most TIR-NB-LRR-type proteins (Aarts et al. 1998; Century et al. 1995; Falk et al. 1999; Glazebrook et al. 1997). An interesting finding is that resistance to *Verticillium albo-atrum* infection in tomato regulated by the receptor-like CC-LRR-transmembrane-PEST R genes *Ve1* and *Ve2* (Kawchuk et al. 2001) is dependent on EDS1. This connects, for the first time, EDS1 activity with membrane-associated recognition proteins (Hu et al. 2005). EDS1, which also, together with PAD4, is required for SA accumulation and the TIR-NB-LRR R gene signaling involving ROI in response to *Hyaloperonospora parasitica* (Feys et al. 2001; Rustérucchi et al. 2001), did not appear to play any major roles in *Arabidopsis* response to *V. longisporum*. In *Arabidopsis*, there are no obvious *Ve* candidates based on sequence analysis, but there is a large family of *Cf2/Clavata1*-like genes. The genes of this family most similar to *Ve* are, however, lacking the PEST sequence found in the *Ve2* gene. The *Ve*-related resistance protein Cf2 in tomato does not require SA for induction of resistance to *Cladosporium fulvum* (Brading et al. 2000), which could explain the dispensability of EDS1/PAD4 for resistance in the *Arabidopsis*-*V. longisporum* interaction. On the other hand, NDR1, which acts as a positive regulator that is required for downstream factors of R-gene resistance (Aarts et al. 1998), was found to be of importance in defense against *V. longisporum* (Fig. 2G). The susceptibility seen on the *ndr1-1* mutant implies that part of the defense system to *V. longisporum* in *Arabidopsis* could be dependent on resistance genes. The *Eix*

Fig. 4. Selected genotypes in salicylic acid (SA), ethylene (ET), and jasmonic acid (JA) signaling pathways pretreated with H₂O, 1-aminocyclopropane-1-carboxylic acid (ACC), methyl jasmonate (MeJA), or salicylic acid (SA) three days before fungal inoculation. Responses are measured as loss of fresh weight in inoculated plants compared with mock-treated plants. The experiment was repeated three times, and standard deviations shown represent between experiment variation. * = significantly different from non-pretreated plants ($P < 0.05$) within each experiment.

genes in tomato, the receptors for the ET-inducing xylanase plant-defense elicitor, have a similar domain organization to the *Ve* genes (Ron and Avni 2004). This finding may suggest corresponding signaling mechanisms in our pathosystem and that early ET induction in *Arabidopsis* in response to *V. longisporum* is dependent on a *Ve*-like recognition.

In order to clarify whether the JA- and ET-associated responses required for resistance acted downstream of or in parallel to *RFO1* or *NDR1*, the *V. longisporum* susceptible mutants *rfo1* and *ndr1-1* were inoculated and assessed after chemical pretreatments. The results showed that pretreatments with MeJA had a strong restoring effect (54% FW loss) on the *ndr1-1* mutant (Fig. 2H) and ACC and SA pretreatments both moderately enhanced *ndr1-1* resistance (63 and 67% FW loss, respectively), compared with water-pretreated control (81% FW loss). Chemical pretreatments of the *rfo1* mutant did not restore resistance. Our interpretation of these results are that the defense hormone-dependent resistance is induced downstream of *NDR1* and is part of a mechanism independent of *RFO1*.

***V. longisporum* causes a switch in expression of SA and JA- and ET-associated PR proteins.**

In a study of the *Arabidopsis*-*V. dahliae* interaction, no transcript of *PR-1* and *PDF1.2*, two common marker genes for SA and JA- and ET-signaling pathways were observed (Veronese et al. 2003), whereas a more recent study found expression of *PR-1*, *PR-2*, and *PR-5* genes (Tjamos et al. 2005). In order to clarify the responses in the *V. longisporum* system, gene expression of the SA marker genes *PR-1* and *PR-2* and the JA and ET markers *PR-4* and *PDF1.2* were assessed on whole plants, 3 and 7 dpi under various pretreatment conditions.

No difference in expression of *PR-1* could be seen between inoculated and uninoculated plants 3 dpi, but the expression of *PR-1* was 2.5-fold higher in inoculated plants compared with the control 7 dpi (Fig. 5A). A challenge with ACC caused a significant increase ($P < 0.05$) of *PR-1* expression in inoculated plants compared with uninoculated plants 3 dpi, whereas a decrease was found in fungal inoculated plants compared with control plants 7 dpi. The shorter time to a response is consistent with previous reports of a potentiating effect on SA by ET (Lawton et al. 1995). The suppression of defense responses at the later stages of infection may reflect a shift in plant defense strategy or that the pathogen can actively suppress defense responses in its host. MeJA treatment induced *PR-1* expression over time. Yet no obvious differences were found between *V. longisporum* and water-inoculated plants. This suggests that MeJA treatment triggers off-target stress responses in the in vitro system used. When the *PDF1.2* expression was monitored, a threefold upregulation was found, both in mock-treated plants and inoculated plants from 3 to 7 dpi (Fig. 5B). A pretreatment with ACC did not cause a significant change of this expression pattern. However, the MeJA pretreatment caused a significant reduction of *PDF1.2* expression at both 3 and 7 days after fungal inoculation, as compared with uninoculated MeJA-treated plants. This unexpected result may be due to *V. longisporum* inducing a negative regulator of JA responses. In the presence of exogenous JA, the effects from the negative regulator become visible. Mock-treated plants did not show any change in the expression pattern of *PR-4* from 3 to 7 dpi, whereas a 5.4-fold increase was observed in the inoculated plants (Fig. 5C). The ACC pretreatment caused a 5.5-fold increase in inoculated plants compared with mock-treated plants at 3 dpi but a 2.2-fold decrease at 7dpi. *PR-4* shows that ET responses are induced early in this pathogen interaction and the expression pattern, when the defense genes are suppressed at later stages of infection, is consistent with what has been observed for *PR-1* and *PDF1.2*. Pretreat-

ment with MeJA induced a 2.5-fold decrease of the expression of *PR-4* in inoculated plants compared with mock-treated plants 3dpi, but no differences could be observed at 7 dpi. However, a 2.9-fold decrease in expression could be seen in healthy plants when compared at 3 and 7 dpi. This result suggests that our MeJA treatment actively suppresses the ET responses after *V. longisporum* infection.

To assess whether *PR-2* was expressed, the transgenic line *PR2::GUS* (Cao et al. 1994) was inoculated with *V. longisporum*. Based on histochemical staining, it was possible to observe extensive expression of β -glucuronidase (GUS) in the midveins of the leaves as well as in the lateral veins in inoculated plants compared with control leaves from 5 dpi and later timepoints (Fig. 6). No GUS activity was observed in the stems or the root of inoculated and control plants. Quantifications of GUS activity resulted in 571 and 3,450 pmol (mg protein)⁻¹ min⁻¹ in plants inoculated with *V. longisporum* 5 and 7 dpi, respectively, followed by a decrease to 1,285 pmol (mg protein)⁻¹ min⁻¹ at 11 dpi (Table 2). These values can be compared with noninoculated control plants, which had a GUS activity of 10 pmol (mg protein)⁻¹ min⁻¹ at day 3 and 260 pmol (mg protein)⁻¹ min⁻¹ at day 7. However, at day 11 only minor differences between control and inoculated plants were observed. The GUS staining results of *PR-2* was thus consistent

Fig. 5. Induction of **A**, *PR-1*, **B**, *PDF1.2*, and **C**, *PR-4* genes in mock- and fungal-inoculated Col-0 plants. Quantification of expression (counts/mm² over local background) was performed via Quantity One. The experiment was repeated three times.

with what had been observed on *PR-1*, *PR-4* and *PDF1.2* slot blot hybridizations after *V. longisporum* inoculation, where the defense responses were suppressed at later stages of the infection. The general trend of a peak expression early in the infection process followed by suppression at later stages implied a switch in strategy, either from the plant or the pathogen.

The importance of flower initiation and abscissic acid (ABA).

The susceptibility of *Arabidopsis* genotypes to *V. dahliae* and *V. longisporum* is phenotypically linked to induction of early flowering (Stevenson et al. 2001; Veronese et al. 2003). A similar observation has been seen on *Brassica napus* plants inoculated by *V. longisporum* (Zhou et al. 2006). Thus, all mutants were also scored for those parameters. Col-0 plants inoculated with *V. longisporum* showed an induction of flowering of 1.3 days earlier compared with control plants. Notably, fungal-inoculated *pad4-1*, *eds4-1*, *npr1-1*, *npr1-3* *ein4-1*, and *jar1-1* genotypes showed a delay in flowering, compared with uninoculated plants, of 3.4, 1.6, 2.4, 1.2, 2.5, and 1.5 days, respectively. This delay restored this character in the mutants, resulting in a similar flowering time to that found in inoculated wild type, possibly due to enhanced SA induction, which has been found to influence flowering time (Martinez et al. 2004). The transition to flowering is a complex process, and several factors, including plant hormones and environmental signals, take an active part. In order to identify an additional important component, a set of mutants impaired in additional hormone signaling was assessed. Interestingly, *aba2-1* showed a very susceptible phenotype and thereby significantly reduced FW

losses as compared with *aba1-3*, *abi1-1*, and *abi4-1* (Fig. 2I). An expected role for ABA under *Verticillium* attack is that the invasion of the xylem should impose a stress similar to drought on the plant. In contrast to the phenotype seen on *aba2-1*, previous studies have revealed a negative interaction between ABA and SA-dependent resistance via negative regulation of phenylalanine ammonia lyase-dependent biosynthesis of SA (Ward et al. 1989). This negative interaction between ABA and SA has been observed from exogenous applications (Audenaert et al. 2002; McDonald and Cahill 1999; Mohr and Cahill 2003; Rezzonico et al. 1998) and endogenous levels of ABA (Audenaert et al. 2002; Thaler and Bostock 2004). In addition to its negative influence on SA-dependent resistance, an antagonistic interaction between ABA and JA and ET signaling modulate defense-gene expression in response to the vascular fungal pathogen *Fusarium oxysporum* was discovered (Anderson et al. 2004). A few observations have recently established a positive role for ABA in pathogen resistance through priming for callose deposition (Ton and Mauch-Mani 2004) and regulation of guard cells against *Sclerotinia sclerotiorum* infection (Guimaraes and Stotz 2004). The callose synthase mutant *pmr4* does not, however, show an enhanced susceptibility in our system, and none of the other ABA mutants compromised in guard cell-dependent resistance against *S. sclerotiorum* revealed enhanced susceptibility to *V. longisporum*. This indicates that this *Verticillium* response is specifically dependent on ABA2 and not a general requirement of the phytohormone ABA.

Impaired root and vascular structures do not influence fungal invasion.

Plant hormones are involved in a range of developmental processes, not least of which is ET, which, for example, takes part in the regulation of flower senescence, premature death, and root hair formation and possibly plays multiple roles in the defense to *V. longisporum*. In order to establish whether pleiotropic effects on development influenced our disease scorings, we assumed that root and vascular structures and chemical compositions of cell walls would be of uttermost importance for successful infection of this fungus. For example lignin, which comprises complex polymers of phenylpropanoid units that are embedded in specialized plant cell walls, such as the secondary wall of xylem cells, have, in form of organic amendments, been shown to contribute to suppressing the viability of *Verticillium* microsclerotia in soil (Debode et al. 2005). Whether the lignin content of the host plants plays a direct role in the defense is unclear. Thus, a range of mutants impaired in those processes was evaluated. Irregular xylem mutants (*irx*) are characterized by degrees of collapsed xylems in stems, most likely through a lack of resistance to the negative pressure exerted by water transport (Turner and Somerville 1997). *irx1-1* has a 50% reduction of cellulose and *irx4-1* has 50% less lignin than wild type (Jones et al. 2001), characters that seriously affect

Fig. 6. Expression of the β -glucuronidase gene under the control of the *BGL2* promoter after treatments with H_2O or inoculation with *Verticillium longisporum* (VI) in Col-0 leaves 3, 5, 7, and 11 days after treatment.

Table 2. Quantification of β -glucuronidase^a

Time ^b	H_2O^c	<i>Verticillium longisporum</i> ^c
1	7.5 $\pm$ 1.5	6 $\pm$ 1
3	10 $\pm$ 1.5	9 $\pm$ 1.5
5	18 $\pm$ 3	571 $\pm$ 15.5
7	260 $\pm$ 19	3450 $\pm$ 34
11	1266 $\pm$ 24	1285 $\pm$ 7

^a Expressed under the control of the *BGL2* promoter after water and *V. longisporum* challenge. The data is based on 20 pooled samples, four plants per pool. The experiment was repeated three times.

^b Days postinoculation.

^c pmol (mg protein)⁻¹ min⁻¹.

cell-wall structures. When the two mutants were assessed 10 days post-*V. longisporum* inoculation, *irx1-1* had lost 70% FW, whereas the corresponding loss of *irx4-1* was 63%. Thus, *irx4-1* was healthier than wild type (*Ler*), which showed a FW loss of 72%. The more-resistant phenotype in *irx4-1* is an effect that might be due to the expansion of the cell walls characteristic of this mutant, suggesting that cellular structures as mechanical barriers have a more important role than pathogen-induced lignification. None of the mutants impaired in root development (*scr2-1*, *scr3-1*, *rhd3-1*, *erh3-1*), two vascular-associated mutants (*gpx1-1* and *vad1*), or a mutant impaired in starch synthesis and onset of flowering (*adg1-1*) showed any differential response to *V. longisporum* as compared with wild type.

The complexity of *Verticillium* wilt-induced responses on *Arabidopsis*.

Various conflicting results are now coming from *Arabidopsis-Verticillium* interaction studies. This can partly reflect the unclear fungal species affiliation. The *V. dahliae* Bob 70 isolate used by Veronese and associates (2003) has lately, both via amplification fragment length polymorphism and sequence analyses, been classified as *V. longisporum* (Fahleson et al. 2003, 2004). Whereas the *V. dahliae* isolate deriving from *Raphanus sativus* is not molecularly characterized (Tjamos et al. 2005). Significant variation exists, however, within *V. longisporum* and *V. dahliae* isolates in their response on *Arabidopsis* (Steventon et al. 2001), which can explain the divergent results. In our work on the *Arabidopsis-V. longisporum* interaction, the complexity of plant-defense responses to this soilborne fungus, in which multiple genetic, developmental, and physiological factors interact and influence disease severity has become evident. This knowledge also highlights different disease-scoring methods used in combination with various developmental stages of the plants. Such variables reflect different genetic components (McDowell et al. 2005). For example, the studies by Tjamos and associates (2005) were performed on soil, and primarily moderate and late disease phenotypes on adult leaves were assessed. This assessment revealed an importance for SA, but in the present study, there are also signs of an early enhanced resistance in SA-deficient plants (8 days after treatment). Our study and Veronese and associates (2003) have focused on an in vitro system, with the obvious difference of screening time. We made the final scoring already after 10 dpi, whereas Veronese and associates (2003) analyzed the plants up to 50 dpi. The more rapid disease progression in our study may explain the stronger dependency of pathogen-defense hormones and early induction of pathogen-response proteins, possibly also due to the different doses of the inoculum used in the two studies. A low inoculum will trigger long-term stress-induced defense responses below detection limit and thus fail to identify the early recognition events that contribute to resistance. On the other hand, a slower disease progression may induce factors required for the recovery of the plant after *Verticillium* wilt infection. Recovery is a mechanism known from particular woody plant species in which *Verticillium* wilt can be overcome by confinement of the fungus and formation of new, uninfected xylem tissue (Hiemstra 1998). Spatial and temporal aspects also influence disease severity and field scorings of crop plants (Dixelius et al. 2005). It is, however, intriguing that the *RFO1* gene regulates resistance to two fungi with similar life styles and infection strategies. Additional similarities between *V. longisporum* and *F. oxysporum* are the dependency of ET, JA, and SA signaling pathways and the *NPR1* gene (Berrocal-Lobo and Molina 2004). Characterization of the additional five *RFO* alleles (Diener and Ausubel 2005), the genetics of the natural variation among

Arabidopsis accessions, and the *lms1* mutant (Bohman et al. 2004) that display enhanced susceptibility to *V. longisporum* have the potential to give us a more comprehensive understanding of the plant defense to vascular wilting diseases.

MATERIALS AND METHODS

Plant material and culture conditions.

Arabidopsis accessions used in the study were Aa-0, Abd-0, Ag-0, Ak-1, An-1, Ba-1, Bay-0, Bch-1, Bd-0, Be-0, Bl-1, Bla-1, Bla-3, Blh-1, Br-0, Bs-1, Bscl-0, Bu-0, Bu-13, Bur-0, C24, Cal-0, Can-0, Cen-0, Cha-0, Chi-0, Chi-1, Cl-0, Co-1, Col-0, Col-4, Ct-1, Cvi-0, Da-0, Db-0, Di-0, Di-1, Do-0, Dr-0, Dra-0, Edi-0, Ei-2, Eil-0, El-0, En-1, Ep-0, Er-0, Es-0, Et-0, Fe-4, Fi-0, Fl-1, Fr-2, Ga-0, Gd-1, Gr-1, Gre-0, Gy-0, Gü-0, Gö-0, Gö-2, Ha-0, Hi-0, Hl-0, Hn-0, Hs-0, In-0, Is-0, Ita-0, Je-0, Jl-1, Jl-2, Jm-0, Kas-1, Kb-0, Kil-0, Kin-0, Kl-0, Kn-0, Kr-0, Kro-0, Kä-0, La-0, Lan-0, Lc-0, Le-0, Ler-0, Li-1, Lip-0, Ll-0, Lm-2, Loh-0, Lu-1, Lz-0, Ma-0, Me-0, Mh-0, Mir-0, Mnz-0, Mr-0, Mrk-0, Ms-0, Mt-0, Mz-0, Na-1, Nc-1, Nd-0, Nie-0, No-0, Nok-0, Np-0, Nw-0, Ob-0, Old-1, Or-0, Ove-0, Oy-0, Pa-1, Per-1, Pf-0, Pi-0, Pla-0, Pn-0, Po-0, Pog-0, Pr-0, Pt-0, Ra-0, Rak-2, Rd-0, Ri-0, Rscl-0, Ru-0, Sah-0, Sap-0, Sav-0, Se-0, Sei-0, Sf-1, Sg-1, Sh-0, Sha-0, Si-0, Sp-0, St-0, Ste-0, Stw-0, Su-0, Ta-0, Te-0, Ts-1, Tsu-0, Tu-0, Tul-0, Ty-0, Uk-1, Wa-1, Van-0, Wc-1, Wei-0, Vi-0, Wil-1, Wl-0, Ws-0, Wt-1, Wü-0, XXX-0, Zü-0, and Zü-1 and the mutants *aba1-3* (Koornneef et al. 1982), *aba2-* and *aba3-2* (Léon-Kloosterziel et al. 1996), *abi1-1*, *abi2-1*, and *abi3-1* (Koornneef et al. 1984), *abi4-1* (Finkelstein 1994), *adg1-1* (Wang et al. 1998), *aux1-7*, *coil-16* (Ellis and Turner 2002), *dnd1-1* (Yu et al. 1998), *eds4-1*, *eds8-1*, *pad4-1* (Glazebrook et al. 1996), *ein2-1*, *ein3-1*, *ein4-1*, *ein6-1* (Guzman and Ecker 1990), *eir1-1*, *eto1-1* (Roman et al. 1995), *etr1-1* (Bleecker et al. 1988), *esal-1* (Tierens et al. 2002), *jar1-1* (Staswick et al. 1992), *pad1-1*, *pad3-1* (Glazebrook and Ausubel 1994), *sid2-1* (Nawrath and Métraux 1999), *eds1-1* (Parker et al. 1996), *erh3-1* (earlier known as *bot1-1*; Bichet et al. 2001), *npr1-1*, *npr1-3* (Cao et al. 1994; Glazebrook et al. 1996), *ndrl-1* (Century et al. 1995), seeds of *NahG* (Lawton et al. 1995), *gpx1-1*, *irx1-1* (*cesa8-1*), *irx4-1* (Turner and Somerville 1997), *pmr4-1* (Vogel and Somerville 2000), *rpr2-4* and renamed to *rar1-13* (Muskett et al. 2002), *rhd3-1* (Schiefelbein and Somerville 1990), *scr2-1* and *scr3-1* (Fukaki et al. 1996), *salk_077975* and *salk_140705* knock-outs of *RFO1* (Diener and Ausubel 2005), and *vad1* (Lorrain et al. 2004). All *Arabidopsis* plant material was grown as described by Steventon and associates (2001).

Fungal isolate, inoculation procedure, and GUS staining.

The *Verticillium longisporum* isolate VD11 was used in the studies (Fahleson et al. 2003; Steventon et al. 2002). *Arabidopsis* plants were inoculated and monitored for three weeks (Steventon et al. 2001). A 100- μ l conidia solution (1.6×10^4 conidia per ml) was added to the surface of 1.5 ml of Murashige and Skoog media (Duchefa Biochemia, Haarlem, The Netherlands) augmented with 1% sucrose (MS-1) in microtiter plates under sterile conditions. Two-week-old seedlings were placed in an upright position in each freshly prepared well. Parameters used to evaluate the plant response were chlorosis formation (number of plants with at least one completely yellow leaf at 10 dpi), loss of FW (average weight reduction per inoculated plant compared with mock-inoculated plants at 10 dpi), stunting, and number of days until the opening of the first flower. Counting of colony-forming units was performed according to Veronese and associates (2003). Histochemical staining and quantification of GUS activity was made using *PR2::GUS* plants 1, 3, 5, 7, and 11 dpi (Cao et al. 1994;

Jeffersson 1987), in which a DyNA Quant 200 fluorometer (Hoefer Pharmacia Biotech, Inc., San Francisco, CA, U.S.A.) was used for the quantifications.

QTL analysis of Bay-0 × Shahdara RIL.

A set of 109 RIL between Bay-0 and Shahdara (Loudet et al. 2002) were grown and inoculated with *V. longisporum* (Stevenson et al. 2001). The formation of chlorosis was scored in the parental lines and RIL 10 dpi. The full set (109 lines) was screened twice, using four to 20 replicates of each line. Analysis of the data was performed in QTL Cartographer (WinQTLCart, North Carolina State University, Raleigh, NC, U.S.A.), using the marker information found on the INRA Versailles' QTL Arabidopsis website.

Pretreatment with ACC, MeJA, and SA.

To study the effect of ET, JA, and SA induction, plants were pretreated with ACC, MeJA, and SA before inoculation with *V. longisporum*. A total of 30 plants of Col-0, *etr1-1*, *jar-1*, *npr1-1*, *npr1-3*, *sid2-1*, *NahG*, *ndr1-1*, and *rfo1* knockouts (salk_077975 and salk_140705) were grown under sterile conditions for 14 days. Seedlings were transferred to fresh solid MS-1 containing 10 µM ACC, 50 µM MeJA, or 100 µM SA (all solved in water, MeJA as an emulsion). All seedlings were fungal-inoculated as described by Stevenson and associates (2001) three days after pretreatment. The weight of inoculated and pretreated plants was related to untreated control plants 10 dpi. The experiment was repeated three times.

Slot-blot hybridization.

For RNA expression, Col-0 seedlings were inoculated by *V. longisporum* and were treated with ACC and MeJA in order to study ET and JA inductions, as described. Seedlings treated with H₂O were used as controls. For each treatment, 48 seedlings were harvested 72 h and 7 dpi after pathogen inoculation, and RNA was extracted using RNeasy (Qiagen, West Sussex, U.K.). The *PR-1*, *PDF1.2*, and *PR-4* genes used as probes are previously described (Penninckx et al. 1996; Potter et al. 1993; Uknes et al. 1992) and were amplified according to Norman-Setterblad and associates (2000). Radioactive labeling of *PR-1* and *PDF1.2* probes was done as described by Bohman and associates (2004), and the *PR-4* probe was radioactive-labeled using random hexamer reaction (Amersham Pharmacia Biotech, Uppsala, Sweden). Blotting (Sambrook and Russell 2001) followed by hybridization and washing of membranes was performed according to the manufacturer's instruction (Amersham Pharmacia Biotech). A Personal FX phosphorimager (Bio-Rad, Hercules, CA, U.S.A.) was used for development of the filters and quantification of expression. Peak volumes were determined through integration under identical areas and background by Quantity One (Bio-Rad) for each filter and probe, respectively.

ET measurements.

Col-0 and *etr1-1* seedlings were grown under sterile conditions. After 14 days they were replanted into 7-ml gas chromatography vials containing 2 ml of MS-1 and 100 µl of conidia solution (1.6×10^4 /ml) of *V. longisporum*. Vials containing uninoculated seedlings of Col-0 and *etr1-1* and 100 µl of distilled water and MS-1 only media with or without *V. longisporum* were included as controls. The released ET from the capped vials was quantified after 1, 3, 5, and 8 dpi, using a Chrompack CP9001 (Varian inc., Palo Alto, CA, U.S.A.) gas chromatograph with a 2.5 m 1/8" HayeSep R coated steel column. The measurements were based on five to ten vials per treatment and genotype, each containing one seedling. The experiment was repeated once.

Statistics.

One-tailed *t*-tests were made to investigate differences between various responses of the *Arabidopsis* genotypes and between treatments performed.

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AUTHOR-RECOMMENDED INTERNET RESOURCE

INRA Versailles' QTL Arabidopsis marker data webpage:
www.inra.fr/qlat/BayxSha/BayxShaMarker.htm