Interfamily Transfer of Tomato *Ve1* Mediates *Verticillium* Resistance in Arabidopsis[1][C][W][OA]

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Vascular wilts caused by soil-borne fungal species of the *Verticillium* genus are devastating plant diseases. The most common species, *Verticillium dahliae* and *Verticillium albo-atrum*, have broad host ranges and are notoriously difficult to control. Therefore, genetic resistance is the preferred method for disease control. Only from tomato (*Solanum lycopersicum*) has a *Verticillium* resistance locus been cloned, comprising the *Ve1* gene that encodes a receptor-like protein-type cell surface receptor. Due to lack of a suitable model for receptor-like protein (RLP)-mediated resistance signaling in Arabidopsis (*Arabidopsis thaliana*), so far relatively little is known about RLP signaling in pathogen resistance. Here, we show that Ve1 remains fully functional after interfamily transfer to Arabidopsis and that *Ve1*-transgenic Arabidopsis is resistant to race 1 but not to race 2 strains of *V. dahliae* and *V. albo-atrum*, nor to the Brassicaceae-specific pathogen *Verticillium longisporum*. Furthermore, we show that signaling components utilized by Ve1 in Arabidopsis to establish *Verticillium* resistance overlap with those required in tomato and include SERK3/BAK1, EDS1, and NDR1, which strongly suggests that critical components for resistance signaling are conserved. We subsequently investigated the requirement of SERK family members for Ve1 resistance in Arabidopsis, revealing that SERK1 is required in addition to SERK3/BAK1. Using virus-induced gene silencing, the requirement of SERK1 for Ve1-mediated resistance was confirmed in tomato. Moreover, we show the requirement of SERK1 for resistance against the foliar fungal pathogen *Cladosporium fulvum* mediated by the RLP Cf-4. Our results demonstrate that Arabidopsis can be used as model to unravel the genetics of Ve1-mediated resistance.

Verticillium wilts caused by soil-borne fungal species of the *Verticillium* genus, of which *Verticillium dahliae* and *Verticillium albo-atrum* are the most common, are devastating vascular plant diseases that occur on a wide host range of over 200 dicotyledonous plant species in temperate and subtropical regions (Fradin and Thomma, 2006; Klosterman et al., 2009). *Verticillium* wilt fungi are notoriously difficult to combat due to extremely persistent resting structures that reside in the soil and that are difficult to eradicate since the only effective control measure, soil fumigation, is expensive and has harmful environmental effects. Furthermore, the broad host ranges of *Verticillium* spp. make crop rotation ineffective, and fungicides to cure infected plants are not available (Wilhelm, 1955; Rowe et al., 1987; Fradin and Thomma, 2006).

Presently, genetic resistance is the preferred method to control *Verticillium* wilt diseases, and *Verticillium* resistance has been described in several plant species (Schaible et al., 1951; Putt, 1964; Huang, 2003; Simko et al., 2004b; Bolek et al., 2005; Zebrowska et al., 2006). However, only from tomato (*Solanum lycopersicum*) has a *Verticillium* resistance locus been cloned (Kawchuk et al., 2001; Fradin et al., 2009). This *Ve* locus mediates resistance against race 1 strains of *V. dahliae* and *V. albo-atrum*, and strains that are not contained by this locus are assigned to race 2 (Schaible et al., 1951; Fradin et al., 2009). The *Ve* locus comprises two genes, *Ve1* and *Ve2*, and although it was initially reported that both *Ve1* and *Ve2* confer resistance when expressed in the close relative potato (*Solanum tuberosum*; Kawchuk et al., 2001), only *Ve1* provides resistance in tomato (Fradin et al., 2009).

Both *Ve1* and *Ve2* encode cell surface receptors that belong to the extracellular leucine-rich repeat class of receptor-like proteins (LRR-RLPs), cell surface receptors with extracellular LRRs that lack a cytoplasmic signaling domain (Kawchuk et al., 2001; Wang et al., 2010a). This class of resistance (R) proteins was identified originally in tomato as Cf resistance proteins that

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provide resistance against the leaf mold pathogen *Cladosporium fulvum* (Jones et al., 1994; Thomma et al., 2005). Furthermore, this class of R proteins includes the apple HcrVf proteins that confer resistance to the scab fungus *Venturia inaequalis* (Vinatzer et al., 2001; Belfanti et al., 2004). In addition to race-specific R proteins, the RLP family harbors receptors that act in basal defense, including the tomato LeEIX receptors for the ethylene-inducible xylanase produced by *Trichoderma* biocontrol fungi (Ron and Avni, 2004) and Arabidopsis (*Arabidopsis thaliana*) AtRLP52 and AtRLP30 that play roles in basal defense against the powdery mildew fungus *Erysiphe cichoracearum* and the bacterium *Pseudomonas syringae* pv *phaseolicola*, respectively (Ramonell et al., 2005; Wang et al., 2008). Finally, AtRLP51 was found to regulate defense against the downy mildew pathogen *Hyaloperonospora arabidopsidis* and *P. syringae* pv *tomato* (Zhang et al., 2010). Apart from defense against pathogens, RLPs also play significant roles in plant development (Wang et al., 2008, 2010a, 2010b).

The interaction between *C. fulvum* and tomato has been the most exploited model to study the genetics of RLP-mediated resistance (Thomma et al., 2005; Wulff et al., 2009), and several components required for the Cf-mediated hypersensitive response or resistance against *C. fulvum* were identified, including the thioredoxin CITRX, the protein kinase ACIK1, the nucleotide binding (NB)-LRR protein NRC1, the U-box protein CMPG1, the mitogen-activated protein kinases LeMPK1, LeMPK2, and LeMPK3, the F-box protein ACRE189/ACIF1, and members of the phospholipase C family (Rivas et al., 2004; Rowland et al., 2005; González-Lamothe et al., 2006; Gabriëls et al., 2007; Stulemeijer et al., 2007; van den Burg et al., 2008; Vossen et al., 2010). Based on their involvement in Cf signaling, a number of candidate genes were tested for a role in Ve1 signaling, revealing that Ve1- and Cf-mediated resistance signaling only partially overlap (Fradin et al., 2009; Vossen et al., 2010). Intriguingly, in addition to NRC1, ACIF1, MEK2, and SERK3/BAK1, both EDS1 and NDR1 were found to be required for Ve1 signaling (Fradin et al., 2009).

Despite the knowledge obtained from tomato, relatively little is known about signaling mediated by RLP-type pathogen receptors (Wang et al., 2010a). This can partially be explained by the lack of a suitable model for RLP-mediated resistance signaling in Arabidopsis, the first plant species for which a genome sequence was released and large mutant collections are available covering nearly every gene in the genome. In the Arabidopsis genome, 57 putative RLP genes (*AtRLP*s) were identified, most of which encode orphan proteins, and attempts to assign biological functions to these genes have met with little success (Ellendorff et al., 2008; Wang et al., 2008). Since Arabidopsis is a host for *Verticillium* infection, we attempted to develop a model for RLP-mediated resistance signaling in Arabidopsis by transfer of the tomato gene encoding Ve1. We show that Ve1 remains fully functional after transfer to Arabidopsis and that *Ve1*-transgenic Arabidopsis is resistant to race 1, but not race 2, strains of *Verticillium*. Furthermore, we show that the signaling components utilized by Ve1 in Arabidopsis to establish *Verticillium* resistance overlap with those that are exploited in tomato (Fradin et al., 2009). We show that the blueprint for resistance signaling is conserved between tomato and Arabidopsis and, thus, that Arabidopsis can be used as a model to unravel the genetics of resistance signaling mediated by the RLP Ve1.

RESULTS

Verticillium Is a Vascular Pathogen of Arabidopsis

Arabidopsis is a widely used model for genetic characterization of disease signaling (Thomma et al., 2001; Nishimura and Dangl, 2010). Although several studies have used Arabidopsis as a host for *V. dahliae* (Veronese et al., 2003; Tjamos et al., 2005; Ellendorff et al., 2009), vascular colonization of Arabidopsis plants has not yet been demonstrated. Therefore, vascular colonization of Arabidopsis roots of the Columbia-0 (Col-0) ecotype by a GFP-transgenic *V. dahliae* strain was studied (Supplemental Materials and Methods S1). Clear GFP signals were observed within xylem vessels (Supplemental Fig. S1), demonstrating that *V. dahliae* is a vascular pathogen of Arabidopsis. This observation was confirmed by plating of stem sections of inoculated and noninoculated plants, showing fungal outgrowth only from stem sections of inoculated plants (Supplemental Fig. S1). This eliminates the possibility that *Verticillium* disease symptoms on Arabidopsis are inflicted by pathogen toxins or effectors that are taken up by the plant in the absence of pathogen colonization.

Comparison of Ve1 with AtRLPs

We queried the Arabidopsis genome for the presence of putative *Ve1* orthologs. Previously, 57 AtRLPs were identified (Wang et al., 2008), of which full-length protein sequences were compared with that of Ve1. Furthermore, the sequences of Ve2, four putative Ve orthologs from *Solanum torvum* (StVe), *Solanum lycopersicoides* (SlVe1), *Mentha longifolia* (MlVe1), and *Mentha spicata* (MsVe1), and tomato Cf-4, Cf-9, LeEIX1, and -2 were added. Phylogenetic analysis demonstrated that Ve1 clusters in a separate clade with Ve homologs from *Solanaceae* as well as non-*Solanaceae* species (Supplemental Fig. S2). Remarkably, none of the 57 AtRLPs clustered with Ve1. Moreover, pairwise amino acid sequence comparison with Ve1 revealed low overall sequence identity between Ve1 and the AtRLPs, with a maximum of only 28% identity between Ve1 and At2g15080 (Supplemental Fig. S2).

Tomato *Ve1* Is Functional against *V. dahliae* and *V. albo-atrum*, But Not against *V. longisporum* in Arabidopsis

In an attempt to develop a model for RLP-mediated resistance signaling, wild-type Arabidopsis plants of

the ecotypes Col-0 and Wassilewskija-0 (Ws-0) were transformed with the tomato *Ve1* coding sequence (CDS) driven by the constitutive cauliflower mosaic virus (CaMV) 35S promoter (*P35S*:*Ve1*; Fradin et al., 2009; Supplemental Figs. S3 and S4). As a control, the 35S-driven tomato *Ve2* CDS (*P35S*:*Ve2*) was used (Fradin et al., 2009). In no case were developmental alterations observed (Fig. 1); subsequently, two transgenic lines were assayed for *Verticillium* resistance. Interestingly, transgenic plants expressing *Ve1* were clearly resistant to *V. dahliae* and *V. albo-atrum* race 1 strains, and only few, if any, symptoms were observed (Figs. 1A and 2A). In contrast, *Ve2* transgenic plants were as diseased as nontransgenic plants upon challenge with these race 1 strains and displayed typical *Verticillium* symptoms, including stunting, wilting, anthocyanin accumulation, chlorosis, early senescence, and necrosis (Figs. 1A and 2B). As expected, *Ve1* and *Ve2*

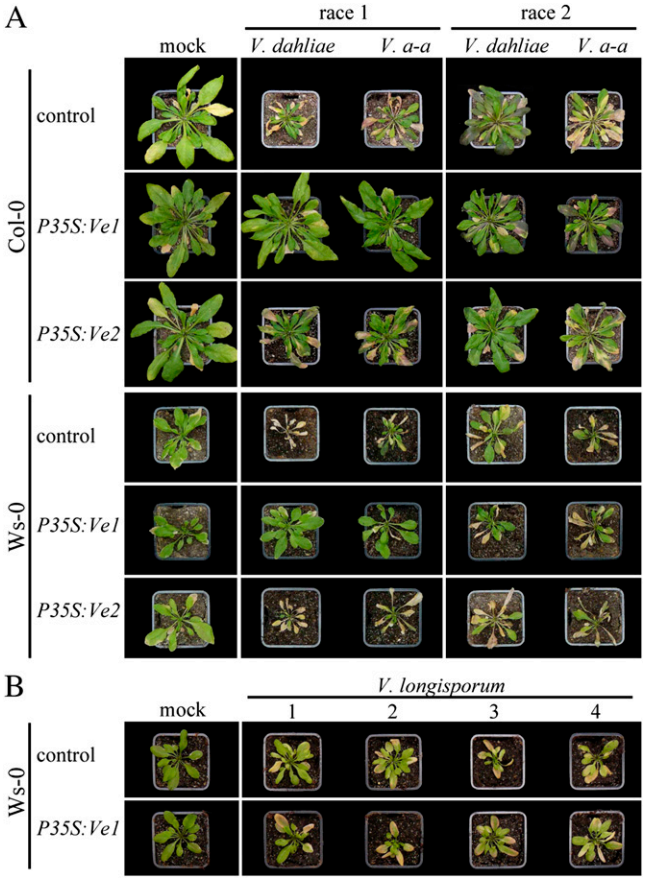

Figure 1. Transgenic expression of *Ve1*, but not of *Ve2*, mediates *Verticillium* resistance in Arabidopsis. Arabidopsis engineered to express tomato CaMV 35S-driven *Ve1* or *Ve2* (*P35S*:*Ve1* and *P35S*:*Ve2*). A, Typical appearance of nontransgenic control and transgenic lines upon mock inoculation or inoculation with race 1 or race 2 strains of *V. dahliae* and *V. albo-atrum* (*V. a-a*) at 21 d after inoculation. B, Typical appearance of nontransgenic Ws-0 control and *P35S*:*Ve1* at 21 d after inoculation with four *V. longisporum* strains (1–4). [See online article for color version of this figure.]

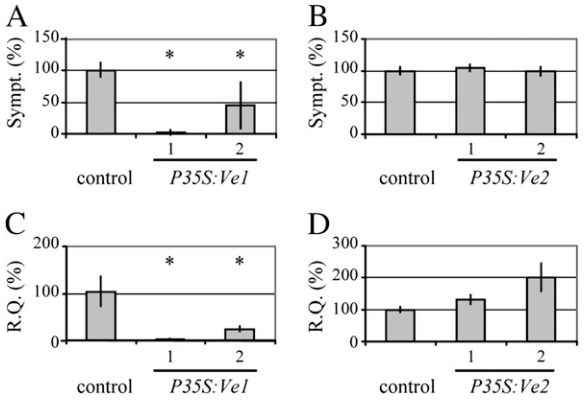

Figure 2. Transgenic expression of *Ve1*, but not of *Ve2*, reduces *Verticillium* wilt symptoms and fungal biomass upon inoculation with *V. dahliae* race 1. Quantification of *Verticillium* wilt symptoms (Sympt.) in Arabidopsis Col-0 engineered to express CaMV 35S-driven tomato *Ve1* (A) or *Ve2* (B) at 21 d after inoculation. Bars represent quantification of symptom development as percentage of diseased rosette leaves with SD. Col-0 (control) is set to 100%. Fungal biomass determined by quantitative real-time PCR (R.Q.) in Arabidopsis Col-0 engineered to express CaMV 35S-driven *Ve1* (C) or *Ve2* (D). Bars represent *Verticillium* ITS transcript levels relative to Arabidopsis Rubisco transcript levels (for equilibration) with SD in a sample of four pooled plants. Col-0 (control) is set to 100%. A to D, Two transgenic lines per construct are shown (1 and 2). Asterisks indicate significant differences when compared with Col-0 ($P < 0.05$).

transgenic lines were fully susceptible when challenged with *V. dahliae* and *V. albo-atrum* race 2 strains (Fig. 1A; Supplemental Fig. S5, A and B). The phenotypes correlated with the degree of *Verticillium* colonization, as determined by real-time PCR (Fig. 2, C and D; Supplemental Fig. S5, C and D). In conclusion, the functionality of *Ve1* and *Ve2* in tomato (Fradin et al., 2009) is fully maintained upon expression in Arabidopsis, as only Ve1 mediates resistance against race 1, but not against race 2 strains of *V. dahliae* and *V. albo-atrum*.

While *V. dahliae* and *V. albo-atrum* are the most prevalent pathogenic *Verticillium* species that occur on broad host ranges, *V. longisporum* particularly infects Brassicaceous hosts (Koike et al., 1994; Karapapa et al., 1997; Barbara and Clewes, 2003). *Ve1*-transgenic plants showed typical *Verticillium* wilt symptoms and were as diseased as wild-type and *Ve2*-transgenic plants when challenged with four different *V. longisporum* strains (Fig. 1B). This suggests that Ve1 does not control *V. longisporum*.

Functional *Ve1* Driven by the Tomato Native Promoter

The functionality of the *Ve1* gene in Arabidopsis was further investigated upon expression driven by the tomato native promoter (*PVe1*:*Ve1*; Fradin et al., 2009; Supplemental Fig. S3). To this end, Col-0 plants were transformed with *PVe1*:*Ve1*, while a similar construct for *Ve2* (*PVe2*:*Ve2*) was used as a control (Supplemental Fig. S3; Fradin et al., 2009). For each construct, two

transgenic lines were challenged with race 1 *V. dahliae*. Plants transgenic for *PVe1*:*Ve1* showed significantly less *Verticillium* wilt symptoms and fungal biomass accumulation when compared with *PVe2*:*Ve2*-transgenic plants and nontransgenic control plants (Fig. 3), showing that the tomato *Ve1* promoter is functional in Arabidopsis. However, the resistance in *PVe1*:*Ve1*-transgenic lines was not as robust as in *P35S*:*Ve1*-transgenic lines as the *PVe1*:*Ve1*-transgenic lines displayed more symptoms and accumulated more fungal biomass than the *P35S*:*Ve1*-transgenic lines (compare Figs. 2 and 3).

Genetic Requirements for Ve1 Signaling in Arabidopsis

The use of Arabidopsis allows exploiting widely available mutant collections. To allow determination of the role of known resistance signaling components in Ve1 signaling, first the role of these components in basal defense against *Verticillium* was evaluated. The genotypes that were used included mutants deficient in salicylic acid (SA) signaling (*eds1-2*, *eds5-1*, *npr1-3*, and *pad4-1*), ethylene (ET) signaling (*ein3-1* and *etr1-1*), jasmonic acid (JA) signaling (*coi1-16* and *jar1-1*), phytoalexin biosynthesis (*pad3-1* and *pad4-1*), and pathogen receptor signaling (*bak1-4*, *eds1-2*, and *ndr1-1*; Supplemental Table S1). All genotypes were challenged with race 1 *V. dahliae* and colonization was determined. When compared with Col-0, no difference in susceptibility was observed for the *coi1-16*, *eds1-2*, *eds5-1*, *eds9-1*, *edr1-1*, *pad3-1*, and *pad4-1* mutants (Fig. 4; Supplemental Fig. S6). However, whereas the mutants *bak1-4*, *jar1-1*, *ndr1-1*, and *npr1-3* showed enhanced *V. dahliae* susceptibility (Fig. 4; Supplemental Fig. S6), the ET mutants *etr1-1* and *ein3-1* showed enhanced resistance (Fig. 4; Supplemental Fig. S6).

To evaluate the contribution of the various signaling components to *Ve1*-mediated signaling in Arabidopsis, all mutants were transformed with the *P35S*:*Ve1* construct (Supplemental Fig. S7). For each mutant, two independent *Ve1*-transgenic lines were challenged with race 1 *V. dahliae* and evaluated for Ve1-mediated disease resistance (Supplemental Fig. S6). To confirm the observed phenotypes, the fungal biomass was determined by real-time PCR in each transgenic line and normalized to the biomass in the respective nontransgenic progenitors to determine the Ve1-mediated biomass reduction. This reduction was then compared to the biomass reduction determined in *Ve1*-transgenic Col-0 when compared with nontransgenic Col-0. This analysis showed that *Ve1*-mediated resistance was not compromised in *edr1-1*, *eds5-1*, *eds9-1*, *ein3-1*, *etr1-1*, *npr1-3*, and *pad3-1* mutants as the *Ve1*-mediated fungal biomass reduction in these mutants was comparable to the reduction in Col-0 (Fig. 5; Supplemental Fig. S6). In contrast, *Ve1*-mediated resistance was compromised in the *bak1-4*, *coi1-16*, *eds1-2*, *jar1-1*, *ndr1-1*, and *pad4-1* mutants as the *Ve1*-transgenic lines showed significantly less fungal biomass reduction when compared to the Ve1-mediated biomass reduction in Col-0 (Fig. 5; Supplemental Fig. S6).

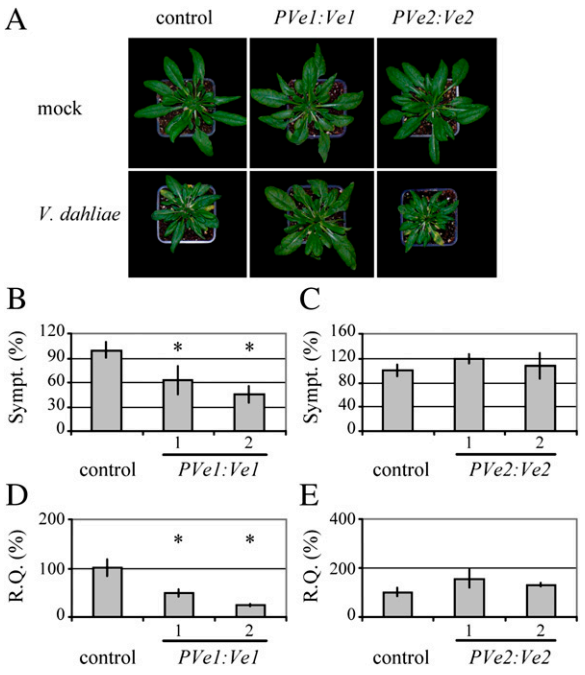

Figure 3. Expression of *Ve1*, but not of *Ve2*, driven by their respective tomato native promoters reduces *Verticillium* wilt disease in Arabidopsis. Arabidopsis Col-0 engineered to express tomato *Ve1* or *Ve2* driven by their respective native promoters (*PVe1*:*Ve1* and *PVe2*:*Ve2*, respectively). A, Typical appearance of nontransgenic Col-0 (control) and transgenic lines upon mock inoculation or inoculation with *V. dahliae* race 1 at 21 d after inoculation. Quantification of *Verticillium* wilt symptoms (Sympt.) in Arabidopsis Col-0 engineered to express tomato *Ve1* (B) or *Ve2* (C). Bars represent quantification of symptom development shown as percentage of diseased rosette leaves with SD. Col-0 (control) is set to 100%. Fungal biomass determined by quantitative real-time PCR (R.Q.) in Arabidopsis Col-0 engineered to express tomato *Ve1* (D) or *Ve2* (E). Bars represent *Verticillium* ITS transcript levels relative to Arabidopsis Rubisco transcript levels (for equilibration) with SD in a sample of four pooled plants. Col-0 (control) is set to 100%. B to E, Two transgenic lines are shown per construct (1 and 2). Asterisks indicate significant differences when compared with Col-0 ($P < 0.05$). [See online article for color version of this figure.]

SERK1 Is Required for Ve1 Signaling in Arabidopsis and Tomato

The somatic embryogenesis receptor-like protein kinase (SERK) was identified in carrot (*Daucus carota*) as a marker for the transition from somatic to embryogenic cells in carrot cell culture (Schmidt et al., 1997). In Arabidopsis, five SERK homologs have been identified with both significantly overlapping and distinct functions (Hecht et al., 2001; Albrecht et al., 2008). Of these five, SERK3 (also known as brassinosteroid insensitive 1 associated receptor kinase 1 [BAK1]) and SERK4 have previously been implicated in pathogen immunity and cell death signaling (Chinchilla et al., 2007; He et al., 2007; Heese et al., 2007; Kemmerling et al., 2007). To investigate whether other SERK family members in addition to SERK3/BAK1 are required for Ve1-medi-

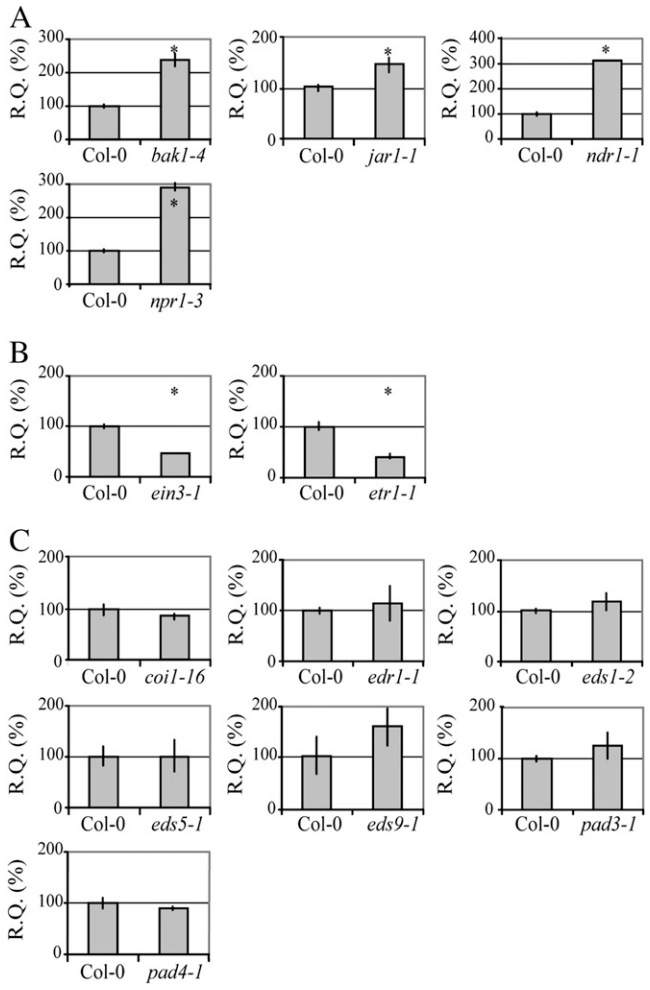

Figure 4. Quantification of *V. dahliae* biomass in Arabidopsis defense signaling mutants. Fungal biomass was determined by quantitative real-time PCR (R.Q.) in Col-0 and defense signaling mutants at 21 d after inoculation. Mutants that show enhanced (A) or reduced (B) susceptibility towards *V. dahliae*. C, Mutants for which fungal biomass is comparable to Col-0. A to C, Bars represent *Verticillium* ITS transcript levels relative to Arabidopsis Rubisco transcript levels (for equilibration) with SD in a sample of four pooled plants. Col-0 is set to 100%. Asterisks indicate significant differences when compared with Col-0 ($P < 0.05$).

ated resistance, we transformed mutants of *Serk1*, *Serk2*, *Serk4*, and *Serk5* with the *P35S*:*Ve1* construct (Supplemental Fig. S8; Supplemental Table S1). For each mutant, two independent *Ve1*-transgenic lines were challenged with race 1 *V. dahliae*, and resistance was evaluated together with the nontransgenic mutants (Fig. 6). To confirm the observed phenotypes, the fungal biomass was determined by real-time PCR in each transgenic line and normalized to the biomass in the respective nontransgenic progenitors to determine the Ve1-mediated biomass reduction. This analysis showed that none of the *Serk* mutants was compromised in basal defense against *Verticillium* as the nontransgenic progenitors showed similar levels of susceptibility as Col-0 plants. Furthermore, *Ve1*-mediated resistance was not compromised in *serk2-1* and *serk5-1* mutants as the *Ve1*-mediated fungal biomass reduction in these mutants was comparable to the reduction in Col-0 (Fig. 6). In contrast, *Ve1*-mediated resistance was compromised in the *serk1-1* mutant and, albeit to a lesser extent and not consistently, possibly also in the *serk4-1* mutant (Fig. 6).

Based on the finding that SERK1 is required for Ve1 signaling in Arabidopsis, we assessed the role of Ve1 signaling in tomato. To this end, the tomato *Serk1* (*SlSerk1*) homolog was identified by BLAST analysis using Arabidopsis SERK1 to query the tomato genome sequence (http://mips.helmholtz-muenchen.de/plant/tomato/index.jsp). One clear *Serk1* homolog was identified (SGN-E623106), and its expression was targeted with virus-induced gene silencing (VIGS; Fradin et al., 2009). To this end, two recombinant tobacco rattle virus (TRV) vectors were designed: one based on the *SlSerk1* CDS and one based on the 3′-untranslated region (3′-UTR). As controls, an empty TRV construct (*TRV*:*00*) and a construct targeting *Ve1* expression were used (Fradin et al., 2009). Subsequently, the recombinant TRV vectors were inoculated onto tomato, and 2 weeks later, half of the plants were inoculated with a race 1 *V. dahliae* strain, while the other half were mock inoculated. Two weeks after inoculation, *Verticillium* resistance was assessed by evaluating the degree of stunting (height of the plant and length of the leaves) as an indicator of disease progression. Upon *Verticillium* inoculation of *TRV*:*00*-treated plants, little stunting was observed when compared with mock-inoculated plants, while *Verticillium* inoculation of *TRV*:*Ve1*-treated plants showed clear and consistent stunting (Fig. 7; Supplemental Table S2). Interestingly, targeting of *SlSerk1* expression also resulted in compromised *Verticillium* resistance, irrespective of whether the CDS or the 3′-UTR was targeted, demonstrating that *Serk1* is required for *Ve1*-mediated *Verticillium* resistance in tomato.

We previously demonstrated that Ve1 signaling only partially overlaps with signaling mediated by Cf proteins that similarly belong to the RLP class of resistance proteins (Fradin et al., 2009; Vossen et al., 2010). To investigate the role of SlSERK1 in Cf signaling, we evaluated the resistance of *Cf-4* tomato plants upon silencing of *SlSerk1* using the construct that targets the UTR and inoculation with a *C. fulvum* strain expressing *Avr4* as well as the constitutively expressed transgenic marker GUS (Vossen et al., 2010). Although no obvious macroscopic disease symptoms were observed, GUS staining clearly indicated colonization of intercellular spaces in the *TRV*:*Serk1*-inoculated plants and not in the control plants (Fig. 7). These histological data strongly suggest that SlSERK1 is required for full Cf-4-mediated resistance.

DISCUSSION

A Model for Ve1 Signaling in Arabidopsis

Resistance to race 1 *Verticillium* strains in tomato is conferred by the single dominant *Ve* locus that was introduced in cultivated varieties in the 1950s (Schaible

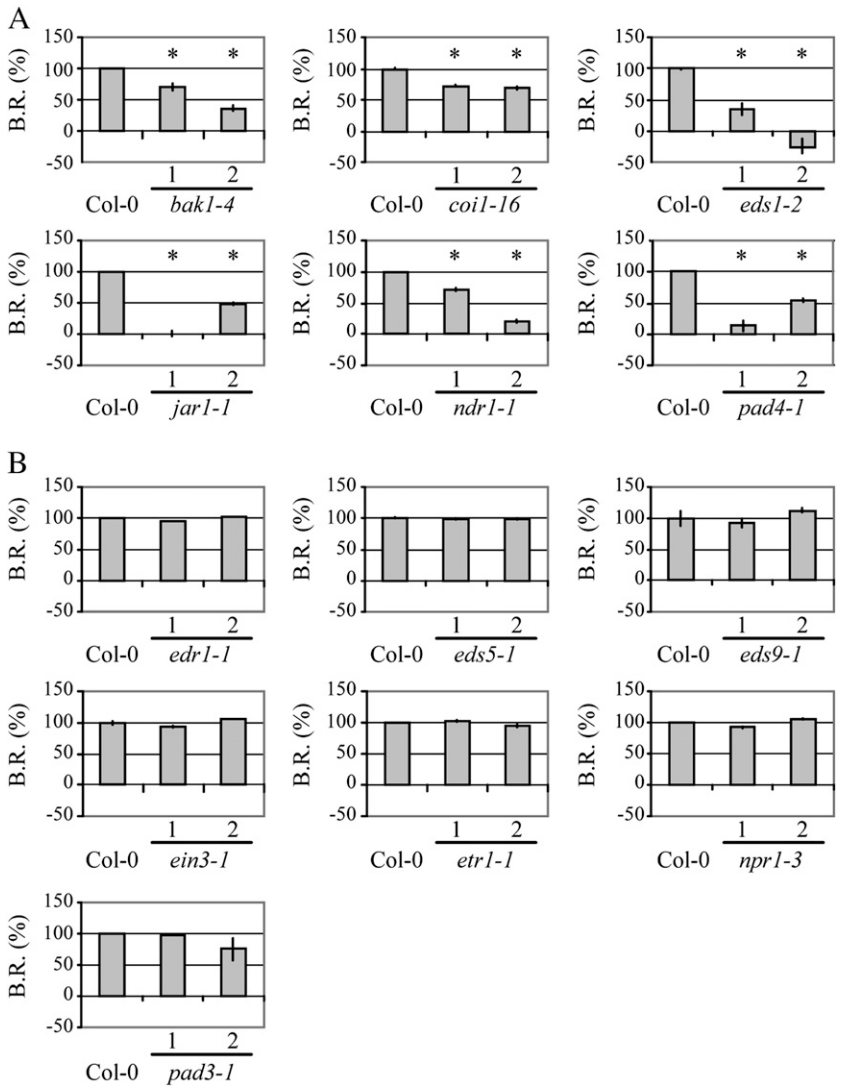

Figure 5. *Ve1*-mediated reduction of *V. dahliae* biomass in defense signaling mutants. Mutants for which *Ve1*-mediated resistance is compromised (A) or not compromised (B). Fungal biomass was determined by quantitative real-time PCR and represents *Verticillium* ITS transcript levels relative to Arabidopsis Rubisco transcript levels (for equilibration). Bars represent the percentage of *Ve1*-mediated fungal biomass reduction (B.R.) in *Ve1*-expressing lines when compared to the fungal biomass accumulated in the respective nontransformed progenitors, with SD in a sample of four pooled plants. *Ve1*-mediated fungal biomass reduction in Col-0 is set to 100%. Two independent transgenic lines expressing *Ve1* are shown per construct (1 and 2). Asterisks indicate significant differences when compared with Col-0 ($P < 0.05$).

et al., 1951) and that is still carried by most commercial tomato varieties. It was reported that both genes that reside in this locus, *Ve1* and *Ve2*, confer resistance against the same race 1 strain when expressed in potato (Kawchuk et al., 2001). However, we subsequently failed to demonstrate a role for *Ve2* in tomato resistance against various race 1 *Verticillium* strains (Fradin et al., 2009). In this study, *Ve1*-transgenic Arabidopsis plants of the Col-0 and Ws-0 ecotypes were found to be resistant to race 1 strains of both *V. dahliae* and *V. albo-atrum* since the plants displayed little to no wilt symptoms and accumulated significantly decreased amounts of fungal biomass when compared with their nontransgenic progenitors. The resistance could not be attributed to generally enhanced pathogen resistance, as *Ve1*-transgenic plants were as susceptible as the control plants towards race 2 strains and four different strains of *V. longisporum*. Furthermore, again we could not confirm a role for *Ve2* in *Verticillium* resistance, as all *Ve2* transgenic lines showed unaltered susceptibility towards *V. dahliae* and *V. albo-atrum*.

The interaction between *C. fulvum* and tomato is the most exploited model to unravel the genetics of RLP-mediated resistance (Thomma et al., 2005; Wulff et al., 2009). In addition, a number of studies have addressed the genetics of Ve1 signaling in tomato (Hu et al., 2005; Fradin et al., 2009; Vossen et al., 2010). These studies have demonstrated that Ve1-mediated resistance signaling only partially overlaps with signaling mediated by Cf proteins. Furthermore, a role for both *Eds1* and *Ndr1* in Ve1 signaling was shown (Hu et al., 2005; Fradin et al., 2009). This is remarkable since differential requirement of *Eds1* and *Ndr1* was shown for cytoplasmic NB-LRR disease resistance proteins in Arabidopsis, as EDS1 generally mediates signaling initiated by Toll and interleukin 1 receptor-NB-LRRs, whereas NDR1 mediates signaling initiated by coiled coil-NB-LRRs (Century et al., 1995; Aarts et al., 1998). Interestingly, we now show that Ve1 requires both *Eds1* and *Ndr1* also in Arabidopsis, thus suggesting that the signaling cascade exploited by Ve1 in Arabidopsis is homologous to the native signaling cascade in tomato.

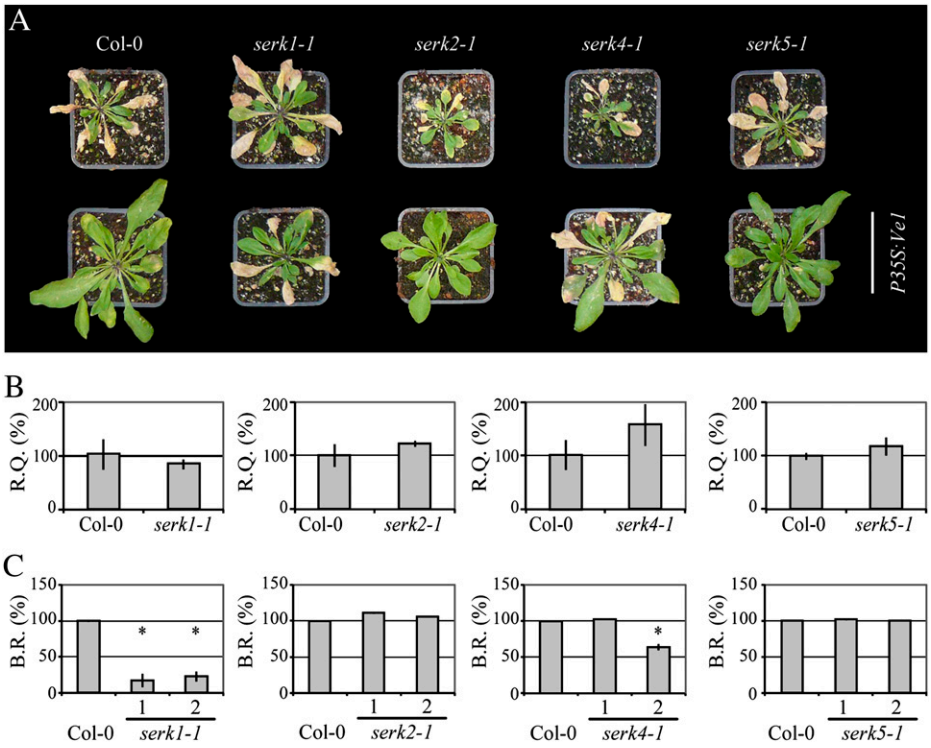

Figure 6. Overview of *Ve1*-transgenic *Serk* mutants challenged with *V. dahliae* race 1. A, Typical appearance of nontransgenic (top row) and *Ve1*-transgenic (bottom row; *P35S*:*Ve1*) Col-0 and *Serk* mutant plants at 21 d after *Verticillium* inoculation. B, Quantification of *V. dahliae* biomass in nontransgenic *Serk* mutants when compared with Col-0. Bars represent *Verticillium* quantification (R.Q.) with SD in a sample of four pooled plants. Col-0 is set to 100%. C, *Ve1*-mediated reduction of *V. dahliae* biomass in *Serk* mutants when compared with Col-0. Bars represent the percentage of *Ve1*-mediated fungal biomass reduction (B.R.) in *Ve1*-expressing lines when compared to the fungal biomass accumulated in the respective nontransformed progenitors, with SD in a sample of four pooled plants. B and C, Fungal biomass was determined by quantitative real-time PCR and represents *Verticillium* ITS transcript levels relative to Arabidopsis Rubisco transcript levels (for equilibration). *Ve1*-mediated fungal biomass reduction in Col-0 is set to 100%. Two independent transgenic lines expressing *Ve1* are shown per construct (1 and 2). Asterisks indicate significant differences when compared with Col-0 ($P < 0.05$). [See online article for color version of this figure.]

This is further supported by the observation that, similar to tomato (Fradin et al., 2009), Ve1 signaling in Arabidopsis requires *Bak1* and does not require *Npr1*.

Basal defense towards *V. dahliae* was compromised in the mutants *bak1-4*, *jar1-1*, *ndr1-1*, and *npr1-3*, whereas the ET mutants *etr1-1* and *ein3-1* showed enhanced resistance. A role for BAK1 in basal defense was previously demonstrated in *Nicotiana benthamiana* and Arabidopsis (Heese et al., 2007; Kemmerling et al., 2007). The finding that *Jar1* and *Npr1* play a role in basal defense cannot be translated into a general requirement of SA and JA for *Verticillium* defense, as other SA and JA signaling components, such as *Eds1*, *Pad4*, and *Coi1*, were not required for *Verticillium* defense. Interestingly, *Ve1*-mediated resistance required the basal defense signaling components *Bak1*, *Jar1*, and *Ndr1*, as well as the components *Coi1*, *Eds1*, and *Pad4*, which are not required for basal defense against *Verticillium*. These findings suggest that JA signaling is required for Ve1-mediated resistance, while SA and ET signaling are not required. We speculated that studies to dissect *Ve1* signaling in Arabidopsis, based on candidate genes and on random mutagenesis, would identify genes that would similarly play a role in *Verticillium* resistance in tomato. To test this hypothesis, we investigated the role of the five Arabidopsis *AtSERK* genes in Ve1-mediated resistance. Over the years, it has become evident that AtSERK family members differentially function in signaling pathways with roles that range from development to defense. While AtSERK1 and AtSERK2 play roles in anther development and male gametophyte maturation (Albrecht et al., 2005; Colcombet et al., 2005), AtSERK1 and AtSERK3/BAK1 act in receptor complexes for brassinosteroid perception (Li et al., 2002; Nam and Li, 2002; Karlova et al., 2006), and AtSERK3 and AtSERK4 have been implicated in programmed cell death responses in development and defense (He et al., 2007; Kemmerling et al., 2007). Here, we show that AtSERK1, AtSERK3, and, to a lesser extent, AtSERK4 are required for full Ve1-mediated resistance in Arabidopsis. We have previously shown that SlSERK3/BAK1 is required for Ve1-mediated resistance in tomato (Fradin et al., 2009), and with VIGS, we now confirm

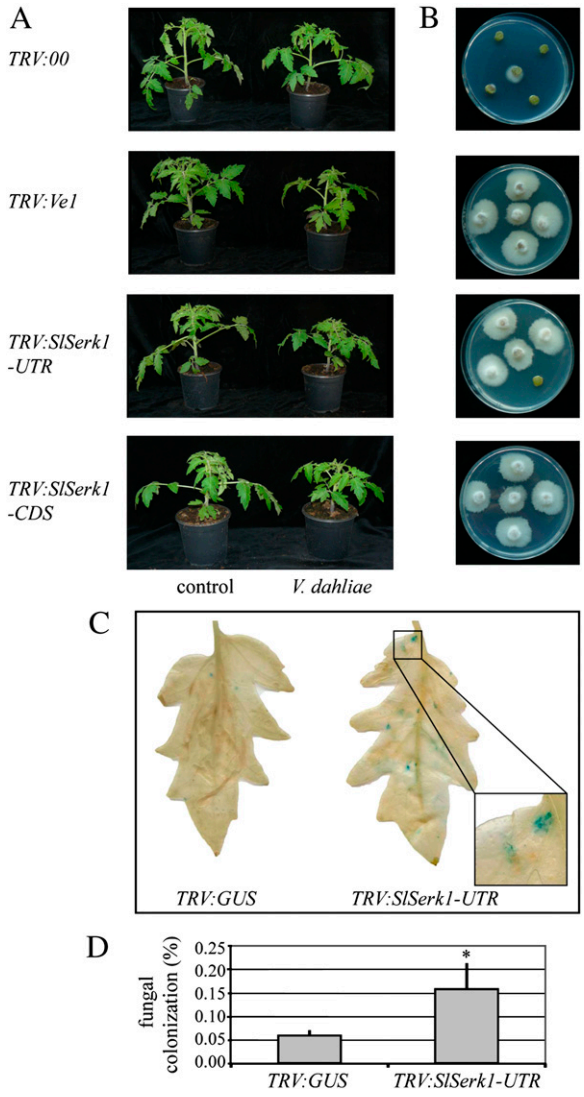

Figure 7. VIGS of *SlSerk1* impairs *Ve1*-mediated *Verticillium* resistance and *Cf-4*-mediated *Cladosporium* resistance in tomato. A, Motelle (*Ve1*/*Ve1*; resistant) plants were treated with an empty recombinant TRV vector (*TRV:00*), a TRV vector targeting *Ve1* (*TRV:Ve1*), the 3′-UTR of *SlSerk1* (*TRV:SlSerk1-UTR*), or the CDS of *SlSerk1* (*TRV:SlSerk1-CDS*). Two weeks after treatment, the plants were mock inoculated (control) or inoculated with a race 1 strain of *V. dahliae*. Photographs were taken 14 d after *V. dahliae* inoculation, and compromised resistance is shown by a stunted appearance of the *V. dahliae*-inoculated plants when compared with mock-inoculated control plants. B, Two weeks after *V. dahliae* inoculation, stem sections were plated, allowing fungal outgrowth as a measure for fungal colonization. Photographs were taken at 14 d after plating. C, *Cf4* tomato plants were treated with a TRV vector targeting GUS (*TRV:GUS*) as a control or a TRV vector targeting the 3′-UTR of *SlSerk1* (*TRV:SlSerk1-UTR*) and challenged with transgenic *C. fulvum* expressing GUS. Representative leaflets after destaining are shown, revealing that full *C. fulvum* resistance is compromised when *Serk1* is targeted. D, Quantitation of fungal growth in *TRV:GUS* and *TRV:SlSerk1-UTR* treated plants. Bars represent the degree of fungal colonization, expressed as the ratio between blue and total leaf area, in leaves from four independent experiments with SE. The asterisk indicates a statistically significant difference ($P < 0.05$). [See online article for color version of this figure.]

that also SlSERK1 is required for resistance in tomato. Together with the recent finding that SlSERK1 is required for aphid resistance mediated by the NB-LRR Mi-1 (Mantelin et al., 2011), our results suggest that SERK1 contributes to host defense mediated by extracellular and cytoplasmic immune receptors and extend the notion that SERK proteins are versatile regulators of various physiological processes in plants. Furthermore, our data demonstrate that *Ve1*-transgenic Arabidopsis can be used as a tool to identify critical signaling components for Ve1 signaling in tomato.

Interfamily Transfer of *Verticillium* Resistance

The transfer of race-specific *R* genes across species boundaries has been mostly successful between phylogenetically related donor and recipient species, while interfamily transfer has generally met little success (Stuiver and Custers, 2001; Hammond-Kosack and Parker, 2003; Gurr and Rushton, 2005; Gust et al., 2010; Wulff et al., 2011). It can be speculated that interfamily transfer of receptors (generally known as pattern recognition receptors [PRRs]) for conserved microbial structures (generally known as pathogen-associated molecular patterns [PAMPs]) could be more successful, as exemplified by the transfer of the Arabidopsis PRRs EFR for bacterial EF-Tu and FLS2 for bacterial flagellin from Arabidopsis to *N. benthamiana* and tomato (Gómez-Gómez and Boller, 2000; Chinchilla et al., 2006; Zipfel et al., 2006; Lacombe et al., 2010). As PRRs are considered to be more ancient than race-specific R proteins, the blueprint of their signaling cascade to establish resistance may be conserved across species and even families, while for R proteins that evolved after speciation, essential signaling components may be lacking. Although *Ve1* is considered to encode a race-specific R protein (Schaible et al., 1951; Kawchuk et al., 2001), several observations support the hypothesis that Ve1 is an ancient pathogen receptor with traits of typical PRRs. First, Ve1-mediated race 1 resistance affects two distinct fungal species, *V. dahliae* and *V. albo-atrum*, suggesting that the yet unidentified elicitor is conserved between species. Second, putative *Ve* orthologs have been identified within (Chai et al., 2003; Fei et al., 2004; Simko et al., 2004a) and outside the *Solanaceae* family (Vining et al., 2007; Vining and Davis, 2009). Third, the receptor-like kinase BAK1/SERK3 that is crucial for various PAMP-triggered responses (Chinchilla et al., 2007; Heese et al., 2007; Kemmerling et al., 2007) is required for Ve1-mediated resistance of tomato (Fradin et al., 2009) and Arabidopsis. Fourth, typical for PRR-mediated resistance, which is generally considered as a weaker variant of R protein-mediated resistance (Tsuda and Katagiri, 2010), Ve1-mediated *Verticillium* resistance is rather weak and allows low-level proliferation of race 1 *Verticillium* strains even in resistant plants (Gold and Robb, 1995; Chen et al., 2004; Fradin et al., 2009). All these observations argue against a role for Ve1 as race-specific R protein and for a role as PRR.

Furthermore, it may be argued that the yet unidentified *Verticillium* activator of Ve1 resistance is a PAMP, as this activator is likely to be conserved across species, reminiscent of Ax21 that is conserved across *Xanthomonas* spp. (Lee et al., 2009). Finally, the functionality of Ve1 after transfer across plant families suggests that it directly recognizes a pathogen component rather than that it guards a host target. This may explain why efforts to identify the Ve1 elicitor, which focused on typical characteristics of effector molecules, have failed thus far.

The current paradigm states that the first line of active plant defense in plant immunity is formed by PRRs that recognize PAMPs and activate PAMP-triggered immunity (PTI). Successful pathogens developed effectors that suppress PTI responses, resulting in effector-triggered susceptibility. Subsequently, some plants developed R proteins to recognize these effectors and activate effector-triggered immunity. Over recent years, several examples illustrate that classifying a particular pathogen molecule as PAMP or effector, or host molecule as PRR or R protein, has become a nebulous exercise (Thomma et al., 2011). The results of this study further illustrate the impossibility to mark Ve1 as PRR or R protein and argue for the existence of a continuum between PTI and effector-triggered immunity.

MATERIALS AND METHODS

All experiments have been performed a minimum of three times yielding similar results.

Plant Material and Manipulations

Plants were grown in soil in the greenhouse or in the climate chamber at 21°C/19°C during 16-h/8-h day/night periods, respectively, with 70% relative humidity and 100 W/m^2 supplemental light when the intensity dropped below 150 W/m^2. Arabidopsis (*Arabidopsis thaliana*) transformations were performed as described (Clough and Bent, 1998). Homozygous single insert transgenic lines were selected by analyzing the segregation of antibiotic resistance. For *Verticillium* inoculations, 2- to 3-week-old Arabidopsis plants were uprooted, and the roots were rinsed in water. Subsequently, the roots were dipped for 3 min in a suspension of 10^6 conidia per milliliter of potato dextrose broth (Difco) and harvested from 1- to 2-week-old *Verticillium* cultures on potato dextrose agar (Oxoid). Control plants were treated similarly, but their roots were dipped in potato dextrose broth without conidiospores. After replanting in fresh soil, disease development was monitored up to 21 d after inoculation. The following strains were used: *Verticillium dahliae* strains JR2 (race 1) and M050414 (race 2), *Verticillium albo-atrum* strains CBS385.91 (race 1) and VA1 (race 2), and *Verticillium longisporum* strains O1, 43, Boc74, and CBS649.85.

In Planta *V. dahliae* Biomass Quantification

Quantification of *V. dahliae* biomass was performed as described previously (Ellendorff et al., 2009). Essentially, 21 d after inoculation, four *V. dahliae*-inoculated plants per genotype were harvested and pooled. The samples were ground to powder, and DNA was extracted from 100 mg of powder. *V. dahliae* biomass was determined by real-time PCR using the qPCR Core kit for SYBR Green I (Eurogentec). To assess *V. dahliae* biomass, the internal transcribed spacer (ITS) region of the ribosomal DNA was targeted using the fungus-specific ITS1-F primer in combination with the *V. dahliae*-specific reverse primer ST-Ve1-R (Supplemental Table S3). For sample calibration, the Arabidopsis large subunit of the Rubisco gene was targeted using the primer pair AtRuBisCo-F3 and -R3 (Supplemental Table S3). Real-time PCR conditions consisted of an initial denaturation step of 10 min at 95°C, followed by denaturation for 15 s at 95°C, annealing for 30 s at 62°C, and extension for 30 s at 72°C for 40 cycles.

Bioinformatic Analysis

The tomato (*Solanum lycopersicum*) Ve1 full-length protein sequence (ACR33105) was compared to the tomato Ve2 sequence (ACR33107; Fradin et al., 2009), the putative Ve homologs from *Solanum torvum* (StVe, AAQ8205), *Solanum lycopersicoides* (SlVe1, AAP20229), *Mentha longifolia* (MlVe1, ACB99682), *Mentha spicata* (MsVe1, ACB99693), Cf-4 (CAA05268), Cf-9 (AAA65235), LeEIX1 (AAR28377), and LeEIX2 (AAR28378) and the 57 AtRLPs (Jones et al., 1994; Thomas, 1997; Chai et al, 2003; Fei et al., 2004; Ron and Avni, 2004; Wang et al., 2008; Vining and Davis, 2009). Multiple sequence alignment and phylogenetic analysis were conducted using *MEGA4* version 4 (Tamura et al., 2007). The multiple sequence alignment was performed using the ClustalW function, using the Gonnet protein weight matrix, a gap opening penalty of 10, and a gap extension penalty of 0.2. The obtained alignment was used as input for the phylogenetic analysis. This analysis was performed using the neighbor-joining method (Saitou and Nei, 1987), p-distance was used as parameter, positions containing alignment gaps were eliminated with the pairwise deletion option, and validity of the analysis was tested by 1000 bootstrap replicates (Felsenstein, 1985). To assess the percentage of protein identity and similarity between tomato Ve1 and the other RLP sequences, the full-length sequences were uploaded in BioEdit. Percentages were calculated based on a pairwise alignment using the Gonnet similarity matrix.

Virus-Induced Gene Silencing followed by Pathogen Inoculation

To amplify the 3′-UTR of *SlSerk1*, 3′-RACE-PCR using the primers SERK1-UTR-F and SERK1-UTR-R (Supplemental Table S3) was used on tomato cDNA. The amplicon was cloned into the pGEMT plasmid (Promega) and transformed to *Escherichia coli*. Plasmid DNA was isolated from single colonies, and the correct *SlSerk1* insert sequence was verified through sequencing. Subsequently, primers UTR-F and UTR-R (Supplemental Table S3) were designed to amplify the 3′-UTR of *SlSerk1* containing *Eco*RI and *Kpn*I restriction sites, respectively. Using the *Eco*RI and *Kpn*I restriction sites, the amplicon was ligated into the TRV2 plasmid (Liu et al., 2002) and subsequently transformed to electro-competent *Agrobacterium tumefaciens* strain GV3101. The TRV construct targeting the SlSERK1 LRR region was generated in a similar fashion using primers SERK1-LRR-F and SERK1-LRR-R (Supplemental Table S3) on tomato cDNA and LRR-F and LRR-R (Supplemental Table S3) to generate the TRV clone. The VIGS procedure followed by inoculation with *V. dahliae* (Fradin et al., 2009) and with *Cladosporium fulvum* (Vossen et al., 2010) was performed as described previously.

To determine the degree of leaf colonization by *C. fulvum*, a transgenic *C. fulvum* strain that constitutively expresses GUS was used. Briefly, leaflets were harvested from inoculated tomato plants, stained with 5-bromo-4-chloro-3-indolyl-β-glucuronic acid, and destained in 70% ethanol to remove natural pigments and facilitate detection of the GUS stain. Images were made from individual leaflets using a flatbed photoscanner, and quantitation of the total leaf area and the total blue area was carried out using the image processing plugin Phenotype Quant of the program ImageJ. The ratio between the total blue area and total leaf area was calculated. Data from four independent experiments were used for statistical analysis.

Supplemental Data

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

Supplemental Figure S1. Vascular colonization of Arabidopsis by GFP-expressing *V. dahliae*.

Supplemental Figure S2. None of the AtRLPs cluster with tomato Ve1.

Supplemental Figure S3. Constructs used for transgenic expression of *Ve1* and *Ve2*.

Supplemental Figure S4. RT-PCR of *Ve1* and *Ve2* expression in transgenic Arabidopsis lines.

Supplemental Figure S5. Transgenic expression of neither *Ve1* nor *Ve2* reduces *Verticillium* wilt symptoms and fungal biomass upon inoculation with *V. albo-atrum* race 2.

Supplemental Figure S6. Overview of *Ve1*-transgenic Arabidopsis defense signaling mutants challenged with *V. dahliae* race 1 at 21 days after inoculation.

Supplemental Figure S7. Phenotypic appearance of mock-inoculated *Ve1*-transgenic Arabidopsis defense signaling mutants.

Supplemental Figure S8. Phenotypic appearance of mock-inoculated *Ve1*-transgenic Arabidopsis *Serk* mutants.

Supplemental Table S1. Arabidopsis mutants used in this study.

Supplemental Table S2. VIGS analysis of *SlSerk1* in resistant Motelle plants.

Supplemental Table S3. Primers used in this study.

Supplemental Materials and Methods S1.

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