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Eggplant and related species are promising genetic resources to dissect the plant immune response to *Pseudomonas syringae* and *Xanthomonas euvesicatoria* and to identify new resistance determinants

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SUMMARY

The apparent lack of durability of many resistance (*R*) genes highlights the need for the constant identification of new genetic sources of resistance for the breeding of new disease-resistant crop cultivars. To this end, we screened a collection of accessions of eggplant and close relatives for resistance against *Pseudomonas syringae* pv. *tomato* (*Pto*) and *Xanthomonas euvesicatoria* (*Xeu*), foliar plant pathogens of many solanaceous crops. Both pathogens caused substantial disease on most genotypes of eggplant and its relatives. Promisingly, however, some of the genotypes were fully or partially resistant to either of the pathogens, suggesting the presence of effective resistance determinants in these genotypes. Segregation of resistance to the growth of *Xeu* following infiltration in F2 progeny from a cross of a resistant and susceptible genotype suggests that resistance to *Xeu* is inherited as a multigenic trait. With regard to *Pto*, a mutant strain lacking all 28 functional type III secreted effectors, and a *Pseudomonas fluorescens* strain expressing a *P. syringae* type III secretion system (T3SS), both elicit a strong cell death response on most eggplant lines. Several genotypes thus appear to harbour a mechanism for the direct recognition of a component of the T3SS. Therefore, eggplant and its close relatives are promising resources to unravel novel aspects of plant immunity and to identify new candidate *R* genes that could be employed in other Solanaceae in which *Xeu* and *Pto* cause agriculturally relevant diseases.

Keywords: *avrptoB*, eggplant, *EtHAN*, *Pseudomonas syringae*, *Solanum melongena*, type III secretion system (T3SS), *Xanthomonas euvesicatoria*.

INTRODUCTION

One of the main approaches to control bacterial plant pathogens is the introduction of new genetic resistance into crops. For example, the introduction of the resistance (*R*) gene *Pto* into tomato controlled outbreaks of bacterial speck disease (Martin *et al*., 1993; Pitblado *et al*., 1984), and the transfer of the *R* gene *Bs2* from pepper to tomato conferred resistance to bacterial spot disease (Tai *et al*., 1999). However, durable genetic resistance has proven to be elusive, as the eventual failure of the *R* genes *Pto* and *Bs2* demonstrates (Gassmann *et al*., 2000; Kunkeaw *et al*., 2010). Indeed, populations of plant pathogens can rapidly shift to overcome plant resistance through the loss or gain of avirulence or virulence components, respectively. Therefore, novel sources of genetic resistance are in constant demand by plant breeders.

In general, active plant defences rely on the specific recognition of pathogen elicitor molecules or their action on plant processes. As the first branch of active immunity, plants detect conserved microbial- or pathogen-associated molecular patterns (MAMPs or PAMPs) via pattern recognition receptors (PRRs) and trigger a complex defence response, known as pattern-triggered immunity (PTI) (Katagiri and Tsuda, 2010). MAMPs are considered to be indispensible for pathogen lifestyle and are thus good targets for immune recognition (Boller and Felix, 2009). Highly conserved regions of the bacterial flagellin protein, for example, contain important MAMPs (Clarke *et al*., 2013; Felix *et al*., 1999).

Adapted pathogens must overcome PTI to cause disease. This is generally accomplished through the deployment of immunity-suppressing effector proteins via (in bacteria) the type III secretion system (T3SS) (Feng and Zhou, 2012). The importance of effectors for pathogen virulence is well demonstrated by the fact that T3SS-deficient mutants of *Pseudomonas syringae* and *Xanthomonas* are severely attenuated in pathogenicity (Hirano *et al*., 1999; Noël *et al*., 2001). The PTI-suppressing ability of effectors is best demonstrated through the actions of the effectors *avrPto1* and *avrPtoB*, which interfere with PTI elicited by bacterial flagellin (Martin, 2012). Indeed, the predominant role of AvrPto1 and AvrPtoB is the suppression of PTI triggered by flagellin, as demonstrated by the fact that deletion of the flagellin-encoding *fliC* gene is sufficient to rescue the virulence defect of an *avrPto1/avrPtoB* double mutant (Kvitko *et al*., 2009). Moreover, these two effectors form the top tier of the *Pto* effector hierarchy, being

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required for full function of all other *P. syringae* pv. *tomato* (*Pto*) effectors in *Nicotiana benthamiana* (Cunnac *et al*., 2011).

In response to pathogen deployment of effectors, plants have evolved R proteins capable of detecting specific effectors, leading to the defence response known as effector-triggered immunity (ETI) (Chisholm *et al*., 2006). Therefore, effectors are double-edged swords for the pathogen: essential for virulence in compatible interactions, but also leading to avirulence in incompatible interactions (Grant *et al*., 2006). The effectors *avrPto1* and *avrPtoB* are again an informative example: both of these effectors are recognized by the plant R protein Pto, ultimately betraying the presence of the harbouring pathogen and leading to avirulence in Pto-expressing plants (Kim *et al*., 2002).

The recognition of specific pathogen effectors by plant R proteins forms the molecular basis for what is classically known as gene-for-gene resistance (Flor, 1971). Although the identification and field deployment of effector-detecting R proteins can provide plant resistance, durability is a major concern with this approach because of the relative ease with which pathogens mutate or drop effector-encoding genes (Dangl *et al*., 2013). Stacking of *R* genes (Halpin and Douglas, 2010) and the deployment of defence genes not involved in effector recognition (Monaghan and Zipfel, 2012) have been suggested as strategies for the creation of more durable forms of resistance in crops. PRRs, for example, have been proposed as another type of defence protein that may provide durable field resistance (Lacombe *et al*., 2010). In this work, we refer to all genetic loci that encode defence-related proteins, including effector-recognizing proteins and PRRs, as *R* loci.

Here, we investigated accessions of eggplant (*Solanum melongena*) and *S. linnaeanum* belonging to the Solanaceae Core Collection of Tomato, Eggplant and Pepper Genotypes (Core-TEP) and included two supplementary resistance sources, *S. melongena* MM127 and *S. aethiopicum Aculeatum* Group MM134. The Core-TEP collection of eggplant is composed of genotypes harbouring resistance to the bacterial wilt pathogen *Ralstonia solanacearum*. We sought to screen this collection for resistance to *Pto*, the causative agent of bacterial speck disease of tomato, and *X. euvesicatoria* (*Xeu*), one of the causative agents of bacterial spot disease of tomato and pepper (Jones *et al*., 2004), and to begin to dissect the molecular basis for resistance and susceptibility of eggplant against these pathogens. We hypothesized that an initial characterization of the pathogenicity and defence responses elicited by bacteria on eggplant and the identification of polymorphic phenotypes within the eggplant collection would identify appropriate germplasm in which to find novel resistance determinants.

Although eggplant was considered to be a host of *Pto* in an older phytopathology manual (Elliott, 1951), neither *Pto* nor *Xeu* is known today to cause any disease in eggplant. Therefore, these genotypes of eggplant and related species might contain potential *R* loci that could be identified and transferred to other Solanaceae on which these pathogens are disease threats. These *R* genes could then provide these crops with novel resistance determinants not previously encountered by these pathogens. Breeders have performed such intra-genus *R*-gene shuttling within Solanaceae successfully in the past (Ano *et al*., 1991; Daunay, 2008).

RESULTS AND DISCUSSION

Pto strain DC3000 (*Pto*DC3000) is pathogenic on eggplant

To determine whether the Core-TEP collection of eggplant genotypes contains a reservoir of *R* genes to control *P. syringae*, we first ascertained the pathogenicity of *Pto*DC3000 (Buell *et al*., 2003). Two accessions, *S. melongena* MM738 and *S. linnaeanum* MM195, whose F2 progeny were used to build the first eggplant linkage map (Doganlar *et al*., 2002; Wu *et al*., 2009), were inoculated via blunt-end syringe infiltration. *Pto*DC3000 population levels were queried 2 and 5 days later. There was a 3.9 log increase in the *Pto*DC3000 population size by 2 days in MM738 and a 2.5 log increase in MM195. By contrast, in the T3SS-deficient *hrcC* mutant of *Pto*DC3000 (Kim *et al*., 2005), there was only approximately a 1 log increase in the population size in both MM738 and MM195, revealing that full growth of *Pto*DC3000 in eggplant depends on a functional T3SS (Fig. 1A). *Pto*DC3000 infection of MM738, but not MM195, led to significant disease symptoms, manifested as leaf collapse in the infiltrated region (Fig. 1B). Following spray infection of MM738, *Pto*DC3000 elicited brown necrotic lesions with yellow halos (Fig. S1A, see Supporting Information) – very similar to the disease symptoms caused by *Pto* on tomato and known as 'bacterial speck'. Spray infection of MM195 with *Pto*DC3000 did not elicit any visible disease symptoms (Fig. S1B). The *hrcC* mutant of *Pto*DC3000 did not elicit any symptoms in either line of eggplant (Fig. 1B). We conclude that *Pto*DC3000 can colonize both MM738 and MM195 in a T3SS-dependent manner, but that MM195 is, at least partially, resistant.

The *Pto*DC3000 effector repertoire is required for full virulence on eggplant

We next employed multiple effector deletion mutant strains of *Pto*DC3000 to better determine the role of effectors in *Pto*DC3000 colonization of eggplant. The D28E deletion mutant strain of *Pto*DC3000, which is missing the 28 functional DC3000 effector genes (Cunnac *et al*., 2011), was severely impaired in growth potential, similar to the *hrcC* mutant. Notably, even a mutant strain missing only the two effectors *avrpto1* and *avrptoB* displayed reduced fitness in colonizing most tested lines of eggplant and related species compared with the wild-type strain (Fig. 1C). This suggests a significant role of these effectors in *Pto*DC3000 colonization of eggplant, and indicates that there is no resistance gene for these two effectors in any of the lines. The effectors

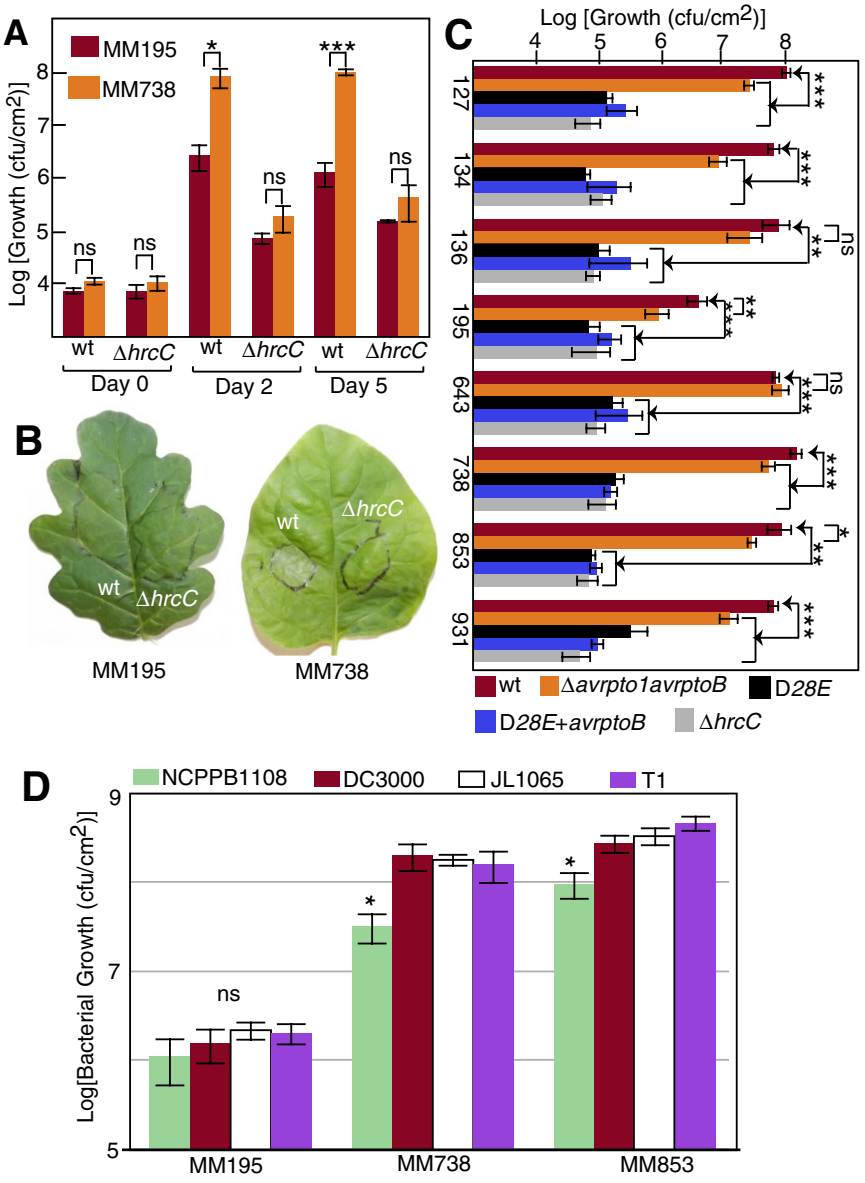

Fig. 1 *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pto*DC3000) is pathogenic on eggplant, dependent on multiple effectors for full pathogenicity and limited in growth on one eggplant line. (A) Population levels of *Pto*DC3000 wild-type (wt) or Δ*hrcC* deletion mutant were quantified 4 h, 2 days and 5 days after infiltration [optical density at 600 nm (OD_{600}) = 0.001] in MM738 and MM195. (B) Representative photographs of disease symptoms from (A) before quantification of population levels on day 2. (C) Population levels of *Pto*DC3000 wild-type (maroon bars), Δ*avrpto1*Δ*avrptoB* mutant (orange bars), D28E mutant (black bar), D28E+*avrptoB* (blue bars) and Δ*hrcC* mutant (grey bars) were quantified 2 days following infiltration (OD_{600} = 0.001) in the indicated accessions. (D) Population levels of four different *Pto* strains were quantified 2 days following infiltration (OD_{600} = 0.001) in the indicated accessions. For all sections, the data represent the mean population levels and error bars represent standard error; $n = 4$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ in a Student's *t*-test. Essentially identical results for all sections were obtained in three independent experiments.

avrpto1 and *avrptoB* have been shown previously to be critical for the colonization of *N. benthamiana* as well (Cunnac *et al*., 2011). However, the T3SS-deficient mutant (Δ*hrcC* here) did not colonize eggplant more efficiently than the D28E mutant strain, in contrast with the observations for *N. benthamiana* (Cunnac *et al*., 2011).

Only a single screened eggplant accession is resistant to *Pto*

Concurrently, we screened other members of the collection of eggplant and close relatives for resistance to *Pto*DC3000 to develop a more detailed pathoprofile of *Pto* on this potential reservoir of resistance sources. As shown in Fig. 1C, MM195 was the only accession on which growth of the pathogen was significantly limited. Population levels of *Pto*DC3000 were at least 1.5 logs higher in the other accessions relative to MM195 2 days following infiltration (statistical analysis in Table S1, see Supporting Information).

To test whether the resistance of MM195 against *Pto*DC3000 is specific to that interaction or comprises broad resistance to other *Pto* strains, we assayed the growth of three other *Pto* strains: NCPPB1108, JL1065 and T1 (Yan *et al*., 2008), on MM195 and two susceptible cultivars, MM738 and MM853. All of the *Pto* strains reached a population size on MM195 that was between 1 and 2 logs lower than on the susceptible cultivars (Fig. 1D), demonstrating that MM195 is broadly resistant to *Pto*. Interestingly, however, *Pto*NCPPB1108 showed less growth compared with the other strains, suggesting reduced virulence of this strain.

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F2 progeny of an interspecific cross suggests the segregation of resistance loci

To preliminarily investigate the genetic architecture of the resistance of MM195 to *Pto*DC3000 compared with the other eggplant lines, we screened F2 progeny from a cross of MM738 × MM195 for susceptibility to *Pto*DC3000 colonization following inoculation by infiltration (note that all F2s and both parents were infiltrated with equivalent ease). Twenty-one F2 plants were as susceptible as MM738 to *Pto*DC3000 infection, whereas 15 showed resistance to colonization similar to MM195 (Table 1), with no intermediate phenotypes. Segregation of resistance and absence of intermediate phenotypes suggests qualitative inheritance instead of quantitative trait loci at the basis of resistance to *Pto*DC3000. The availability of a map of this cross (Doganlar *et al*., 2002) should make it feasible to map and clone the underlying locus/loci in the future.

*Pto*DC3000 elicits effector-independent rapid cell death in eggplant

To screen for the possible presence of ETI as a result of the recognition of *Pto*DC3000 effectors by eggplant resistance genes, we screened the eggplant lines for rapid cell death indicative of a hypersensitive response (HR) after high-dose infiltration [optical density at 600 nm (OD_{600}) = 0.1] with *Pto*DC3000 and various effector deletion mutants. Unexpectedly, wild-type *Pto*DC3000 elicited strong rapid cell death on the highly resistant accession MM195, as well as on all susceptible accessions (Table 2). Therefore, the cell death is probably not representative of a true HR. Representative images of the rapid cell death elicited by infiltration are shown in Fig. S2 (see Supporting Information). Intriguingly, even *Pto*DC3000D28E elicited rapid cell death on the majority of eggplant lines (Table 2). As *Pto*DC3000D28E does not reach a high population density in these accessions (see above), the observed leaf collapse response appears to be a defence response and is not simply collapse caused by high pathogen load. Moreover, *Pto*DC3000D28E elicits rapid cell death in MM738, even after infiltration with only one-tenth of the bacterial load, OD_{600} = 0.01 (Fig. S3A,B, see Supporting Information). Only accession MM134 does not exhibit significant rapid cell death following *Pto*DC3000D28E infiltration (Table 2). Importantly, even 2 days following *Pto*DC3000D28E infiltration, MM134 does not exhibit macroscopic cell death (Fig. S3A,B), further demonstrating that components of the pathway responsible for the cell death response observed in the other accessions are missing in MM134. The T3SS-deficient *hrcC* mutant strain did not elicit macroscopic cell death in any eggplant line (Table 2).

As the D28E mutant is neutered of all expressed effectors, the only functional difference between this strain and the Δ*hrcC* mutant strain is the presence of the T3SS machinery and associated helper proteins, such as harpins, in *Pto*DC3000D28E (Cunnac *et al*., 2011). We therefore hypothesize that a component of the T3SS, or an associated helper protein, is the elicitor of the cell death response observed in the eggplant genotypes, except for MM134. It is thus surprising that *Pto*DC3000Δ*hrcC* does not grow more strongly than *Pto*DC3000D28E on any eggplant accession, and that *Pto*DC3000D28E does not grow more strongly on MM134 than on other eggplant accessions (Fig. 1C). Moreover, because the T3SS-dependent, effector-independent cell death response occurs in the majority of the eggplant genotypes susceptible to *Pto*DC3000 infection, we cannot correlate T3SS recognition with resistance. At present, the contribution of the rapid cell death elicited by *Pto*DC3000D28E towards defence is unclear.

Interestingly, we found that the effector AvrPtoB reduces significantly the rapid cell death elicited by *Pto*DC3000D28E in most tested eggplant accessions when expressed in the *Pto*DC3000D28E background (Table 2). Therefore, AvrPtoB does not trigger cell death in eggplant. Because AvrPtoB is known to interfere with programmed cell death (Abramovitch and Martin, 2005) and to target several surface-localized PRRs for inhibition or degradation (Gimenez-Ibanez *et al*., 2009; Göhre *et al*., 2008; Zeng *et al*., 2012), this result further suggests that the observed rapid cell death is a kind of programmed cell death, or that the perception of T3SS components (e.g. harpin) relies on surface-localized immune receptors which are targeted by AvrPtoB. Notably, the expression of *avrPtoB* was less effective at suppressing the cell death response in the *Pto*DC3000-resistant MM195 genotype (Table 2, Fig. S2), suggesting that suppression of the elicited cell death response by AvrPtoB is correlated with virulence.

To confirm that the machinery of the T3SS itself is eliciting the rapid cell death response, we infiltrated either wild-type *Pseudomonas fluorescens* (*Pf*) 0-1 or the same strain engineered to express a *P. syringae* T3SS, called EtHAN (Thomas *et al*., 2009), into multiple accessions. EtHAN, but not wild-type *Pf* 0-1, elicits strong macroscopic cell death on all tested accessions except,

Table 1 Segregation pattern of susceptibility to *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pto*DC3000) in F2 progeny of an MM195 × MM738 cross.

Plant	Resistant*	Susceptible†
MM195	6	0
MM738	0	6
F2 MM195 × MM738	15	21

*Resistant plants are defined as having population levels statistically identical ($P < 0.05$ in a Student's *t*-test) to MM195 plants in side-by-side infection. These plants never portrayed substantial macroscopic cell death in the infiltration area. †Susceptible plants are defined as having population levels statistically identical ($P < 0.05$ in a Student's *t*-test) to MM738 plants in side-by-side infection. These plants always portrayed substantial macroscopic cell death in the infiltration area.

Table 2 Elicitation of rapid cell death symptoms of wild-type *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pto*DC3000) and selected mutant lines in the collection of eggplant and close relatives. Mean cell death score (0, 0–15% leaf collapse; 1, 16–30% leaf collapse; 2, 31–70% leaf collapse; 3, 71–100% leaf collapse) of six individual leaves; the standard error is given in parentheses. Letters represent statistical groupings of mean cell death scores across the mutant strains of *Pto*DC3000 in a Student's *t*-test, $P < 0.05$. Similar results were obtained in three independent experiments.

Plant material	Wild-type	*Δavrpto1ΔavrpotB*	D28E	D28E+*avrptoB*	*ΔhrcC*
MM127	2.75 (0.5) A	3 (0) A	2.75 (0.5) A	0.5 (0.58) B	0 B
MM134	3 (0) A	3 (0) A	0.25(0.25) B	0.25 (0.25) B	0 B
MM136	3 (0) A	3 (0) A	2.25 (0.25) B	1 (0.41) C	0 D
MM195	3 (0) A	3 (0) A	3 (0) A	2 (0.41) B	0 C
MM643	3 (0) A	3 (0) A	2 (0.41) B	0.5 (0.5) C	0 C
MM738	2.5 (0.57) A	2.5 (0.57) A	0.81 (0.41) B	0.75 (0.48) B	0.25 (0.25) B
MM853	2.75 (0.5) A	2.75 (0.5) A	1.25 (0.25) B	0.75 (0.25) B	0.5 (0.29) B
MM931	2.75 (0.5) A	3 (0) A	3 (0) A	1.5 (0.29) B	0.25 (0.25) C

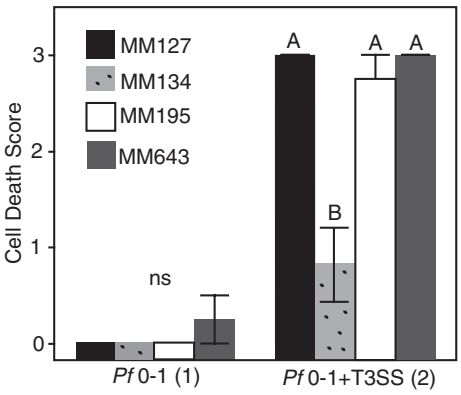

Fig. 2 *Pseudomonas fluorescens* (*Pf*) 0-1 expressing the *Pseudomonas syringae* type III secretion system (T3SS) elicits rapid cell death on accessions in a plant genotype-dependent manner. Plants were infiltrated with either *Pf* 0-1 wild-type or *Pf* 0-1 with a *P. syringae* T3SS at an optical density at 600 nm (OD_{600}) = 0.1 and scored 24 h later. Data bars represent mean cell death scores (0, no leaf collapse; 3, total leaf collapse) and error bars represent standard error; $n = 6$. Similar results were obtained in three independent experiments. Letters represent statistical groupings of mean cell death scores in a Student's *t*-test, $P < 0.05$.

again, MM134 (Figs 2, S4, see Supporting Information). At lower doses, EtHAN does not trigger strong cell death on MM738 (Fig. S3C,D), in contrast with the lower dose infections of MM738 with *Pto*DC3000D28E. MM134 does not respond with strong cell death following EtHAN treatment, even 3 days following infiltration (Fig. S3C,D), again suggesting that MM134 is missing loci responsible for this cell death response. Expression of the *P. syringae* T3SS in *Pf* 0-1 also led to rapid cell death in tomato (*S. lycopersicum* cv. Rio Grande) and cultivated tobacco (*N. tabacum* cv. Burly), but not Arabidopsis (*A. thaliana* ecotype Col-0) or *N. benthamiana*, apart from a few leaves with minor leaf collapse (Fig. S5, see Supporting Information), which is consistent with the initial characterization of the EtHAN strain (Thomas *et al*., 2009).

Recognition of the T3SS by *N. benthamiana* has been proposed previously following the observation that a T3SS-deficient strain grew better than *Pto*DC3000D28E (Cunnac *et al*., 2011), and components of the T3SS have been shown previously to elicit an immune response in *N. benthamiana* (Oh *et al*., 2010). Because we found that, in our conditions, EtHAN triggers HR-like symptoms on *N. tabacum*, but not *N. benthamiana*, we also tested the ability of wild-type *Pto*DC3000 and *Pto*DC3000D28E to elicit macroscopic cell death on *N. tabacum* and *N. benthamiana*. *Pto*DC3000D28E did not elicit macroscopic cell death on either of these hosts (Fig. S6, see Supporting Information). Therefore, our assays were unable to confirm any HR-like elicitation as a result of the T3SS itself in *N. benthamiana*. We hypothesize that, under our conditions, T3SS triggered cell death in *N. tabacum* when expressed in the *Pf* 0-1 background, but not in *Pto*DC3000D28E, because *Pto*DC3000D28E possesses a certain factor capable of suppressing this plant defence response, possibly the phytotoxin coronatine, a methyl-jasmonate mimic that suppresses many plant defences against *Pto* (Nomura *et al*., 2005). Alternatively, allelic differences between the T3SS of *Pto*DC3000 and T3SS of *Psy*61 (which is the T3SS expressed in EtHAN) could explain this non-congruent result.

Taken together, these results suggest that eggplant and a subset of other Solanaceae are equipped with a receptor for a component of the T3SS or T3SS helper proteins. The T3SS helper proteins HrpW1 and HrpZ (also called harpin) are both known to elicit an HR *in planta* (Alfano *et al*., 1996; Charkowski *et al*., 1998). However, only *hrpZ* is present in both *Pto*DC3000D28E and EtHAN. As evidence for a HrpZ receptor has been proposed previously in tobacco (Lee *et al*., 2001), HrpZ is also a strong candidate for the elicitor in eggplant. The difference among eggplant accessions in responding with rapid cell death to both *Pto*DC3000D28E and EtHAN provides an excellent tool to elucidate the underlying genetic pathway in the future.

Xanthomonas is pathogenic on eggplant

Because *Xeu* represents another major bacterial threat to several solanaceous crops, we also sought to establish a pathoprofile of *Xeu* on the accessions of eggplant and relatives. We initially queried whether strain VT2281 [isolated during a bacterial spot

outbreak on tomato in Virginia and identified by multilocus sequence typing (MLST) (Almeida *et al*., 2010) as *Xeu*] can colonize eggplant by infiltrating both *S. melongena* MM738 and *S. linnaeanum* MM195. *Xeu*VT2281, although slower than *Pto* at colonizing eggplant, aggressively colonized MM738, with a more than 3.4 log increase in population size by 8 days following infiltration. MM195 was instead resistant to *Xeu*VT2281 colonization, supporting only an approximately 1.4 log increase in population size through 8 days (Fig. 3A). In addition, no disease symptoms developed in MM195 following *Xeu*VT2281 infiltration, in contrast with considerable disease symptoms that developed in MM738 (Fig. 3B).

We then screened the other accessions for resistance to *Xeu*VT2281 colonization. In total, three accessions were highly susceptible to *Xeu*VT2281, four were very resistant and two had an intermediate phenotype (Fig. 3C). In general, disease symptom severity correlated with *Xeu*VT2281 population levels (Figs 3B and S7, see Supporting Information).

*Xeu*VT2281 does not elicit HR-like macroscopic cell death on any tested accession

Considering that ETI is a potential explanation of the resistant interactions and is often visible in resistant plants as an HR, we screened the collection of eggplant lines for HR-like responses to high-dose *Xeu*VT2281 inoculation. We specifically hypothesized that *Xeu*VT2281 would trigger an HR on the resistant eggplant lines, suggesting ETI. However, wild-type *Xeu*VT2281 did not elicit any macroscopic cell death on any of the eggplant lines (Fig. S8, see Supporting Information). We therefore conclude that the plant defence response pathways responsible for resistance to *Xeu*VT2281 are not dependent on an HR-like response, or that *Xeu*VT2281 can efficiently suppress any HR-like response as a result of the secretion of HR-suppressing effectors, even in resistant interactions.

Multiple quantitative trait loci (QTLs) are associated with the resistance of eggplant to *Xeu*VT2281

Finally, we performed a preliminary *Xeu*VT2281 colonization screen on the F2 progeny from the interspecific cross of the resistant *S. linnaeanum* (MM195) with the susceptible *S. melongena* (MM738). The individual F2s exhibited both extreme and several intermediate phenotypes in resistance to *Xeu*VT2281 colonization (Fig. 4). These preliminary results suggest that the resistance of MM195 to *Xeu*VT2281 is probably encoded by several QTLs.

CONCLUSIONS

Pathogenicity screens of a collection of accessions of eggplant and relatives allowed the rapid identification of the genotypes susceptible and resistant to two bacterial plant pathogens, *Pto* and *Xeu*. In addition, all but one of the tested eggplant accessions appeared to recognize a component of the T3SS of *Pto*DC3000. Although this recognition does not correlate with resistance in eggplant, the responsible receptor may be effective in improving resistance

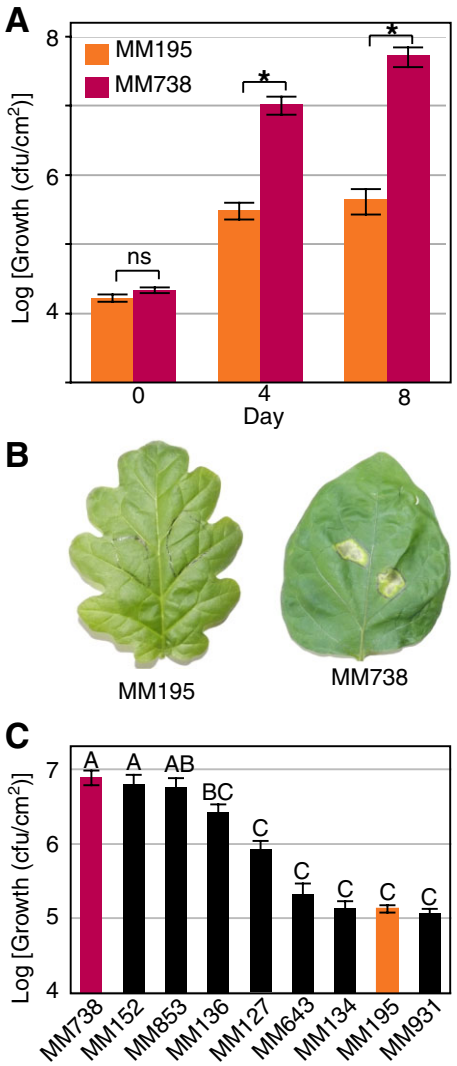

Fig. 3 *Xanthomonas euvesicatoria* (*Xeu*) is pathogenic on eggplant line MM738, but not MM195. (A) Population level of *Xeu*VT2281 was quantified 8 days after infiltration [optical density at 600 nm (OD_{600}) = 0.001] into MM738 or MM195. Data bars represent mean population levels of *Xeu*VT2281 and error bars represent standard error; $n = 4$. *$P < 0.05$ in a Student's *t*-test. (B) Representative photographs of disease symptoms before quantification of population levels on day 8. (C) Population level of *Xeu*VT2281 was quantified 8 days after infiltration (OD_{600} = 0.001) into the indicated accessions. Data bars represent mean population levels of *Xeu*VT2281 and error bars represent standard error; $n = 4$. Letters indicate statistical groupings, $P < 0.05$ in a Student's *t*-test. Essentially identical results were obtained in three independent experiments for all sections.

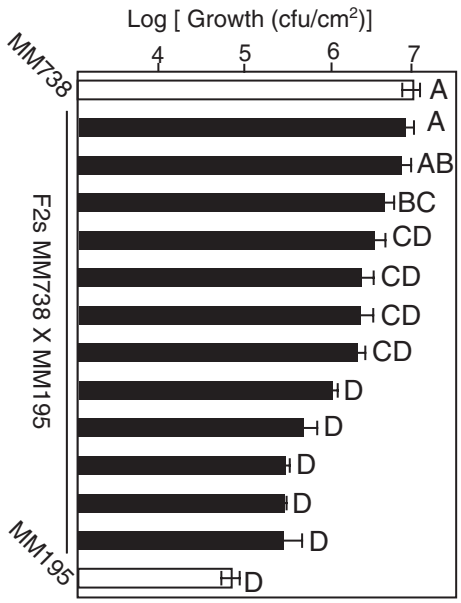

Fig. 4 Infection of the F2 progeny of resistant and susceptible accessions with *Xanthomonas euvesicatoria* strain VT2281 (*Xeu*VT2281) reveals resistance segregation. The population level of *Xeu*VT2281 was quantified 8 days after infiltration [optical density at 600 nm (OD_{600}) = 0.001] in eggplant lines MM738 (susceptible), MM195 (resistant) and F2 progeny of MM738 × MM195. Data bars represent mean population levels of *Xeu*VT2281 and error bars represent standard error; $n = 4$. Letters represent statistical groupings of mean population levels in a Student's *t*-test, $P < 0.05$.

in other crops, and/or homologues of the receptor present in other plant species may be more efficient in conferring disease resistance.

Because we observed segregation of resistance to *Xeu* and *Pto* in an F2 population of two eggplant accessions and recognition of the T3SS of *Pto* is absent in one accession, we conclude that the eggplant accessions characterized here can be used to map and clone new *R* loci. This can be expected to allow new insight into the basic mechanisms of plant immunity in a so far poorly characterized crop species. Moreover, the identified loci can consecutively be deployed in the closely related crops tomato and pepper, on both of which *Xeu* and *Pto* cause economically important diseases.

EXPERIMENTAL PROCEDURES

Plant and bacterial growth conditions

Seeds were sown in Miracle-Gro moisture control potting mix in a Conviron (Winnipeg, MB, Canada) ATC40 chamber at 22 °C, 65% humidity, 12-h light cycle for 7–9 weeks after germination before use. All bacterial strains were grown at 28 °C for 20–28 h on King's B medium (KB) plates with appropriate antibiotics (50 μg/mL rifampicin for *Pto* and 50 μg/mL kanamycin for *Xeu*) before use. *Pto*DC3000D28E and *Pto*DC3000 Δ*avrpto1*Δ*avrptoB* were kindly provided by Alan Collmer (Cornell University, Ithaca, NY, USA). Strains *Pf* 0-1 and EtHAN were kindly provided by Jeff Chang (Oregon State University, Corvallis, OR, USA). The broad-host-range plasmid pVSP61 (kindly provided by Bingyu Zhao, Virginia Tech, Blacksburg, VA, USA) was transformed into strain VT916 to create the kanamycin-resistant strain *Xeu*VT2281. VT816 was isolated from a tomato plant on the eastern shore of Virginia during a bacterial spot outbreak, and identified as *X. euvesicatoria* by MLST.

Plant infection assays

Bacterial strains were streaked onto KB plates containing appropriate antibiotics and grown at 28 °C for 20–28 h. Bacteria were then resuspended in 10 mM $MgSO_4$ and diluted to $OD_{600} = 0.001$. These bacterial suspensions were then infiltrated into leaves of 7–9-week-old eggplants using a blunt-end syringe. Leaf punches ($n = 4$, taken from four different leaves from two different plants) were collected from infiltrated areas, 2 days (for *Pto*) or 8 days (for *Xeu*) later using a 4-mm cork borer, and homogenized in microtitre tubes containing 200 μL of 10 mM $MgSO_4$ and three 2-mm glass beads using a Mini BeadBeater (Biospec Products, Bartlesville, OK, USA). Homogenized samples were diluted and plated on KB plates with appropriate antibiotics, and colony-forming units (cfu) were counted and used to calculate cfu/cm^2 of plant tissue.

Rapid cell death assays

Rapid cell death assays were performed on plants 7–9 weeks after planting. Bacterial strains were propagated on selective KB media. After 20–28 h of growth, bacteria were suspended in 10 mM $MgSO_4$ and diluted to the desired OD_{600} (0.1 for all eggplant accessions, *N. benthamiana* and *N. tabacum*; 0.3 for tomato and Arabidopsis). For *Pto*DC3000, $OD_{600} = 0.1$ corresponds to $\sim 7.7 \times 10^7$ cfu/mL and $OD_{600} = 0.3$ corresponds to $\sim 2.6 \times 10^8$ cfu/mL. For *Xeu*VT2281, $OD_{600} = 0.1$ corresponds to $\sim 2.2 \times 10^8$ cfu/mL and OD = 0.3 corresponds to $\sim 6.1 \times 10^8$ cfu/mL. Leaves were inoculated with bacterial strains via blunt-end syringe infiltration. Leaf appearance was scored 24 h after infiltration and rated on a scale from '0' to '3' ('0' representing no cell death and '3' representing complete leaf collapse within the infiltration site).

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REFERENCES

Abramovitch, R.B. and Martin, G.B. (2005) AvrPtoB: a bacterial type III effector that both elicits and suppresses programmed cell death associated with plant immunity. *FEMS Microbiol. Lett.* **245**, 1–8.

Alfano, J.R., Bauer, D.W., Milos, T.M. and Collmer, A. (1996) Analysis of the role of the *Pseudomonas syringae* pv. *syringae* HrpZ harpin in elicitation of the hypersensitive response in tobacco using functionally non-polar hrpZ deletion mutations, truncated HrpZ fragments, and hrmA mutations. *Mol. Microbiol.* **19**, 715–728.

Almeida, N.F., Yan, S., Cai, R., Clarke, C.R., Morris, C.E., Schaad, N.W., Schuenzel, E.L., Lacy, G.H., Sun, X., Jones, J.B., Castillo, J.A., Bull, C.T., Leman, S., Guttman, D.S., Setubal, J.C. and Vinatzer, B.A. (2010) PAMDB, a multilocus sequence typing and analysis database and website for plant-associated microbes. *Phytopathology*, **100**, 208–215.

Ano, G., Hebert, Y., Prior, P. and Messiaen, C. (1991) A new source of resistance to bacterial wilt of eggplants obtained from a cross: *Solanum aethiopicum* L × *Solanum melongena* L. *Agronomie*, **11**, 555–560.

Boller, T. and Felix, G. (2009) A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* **60**, 379–406.

Buell, C.R., Joardar, V., Lindeberg, M., Selengut, J., Paulsen, I.T., Gwinn, M.L., Dodson, R.J., Deboy, R.T., Durkin, A.S., Kolonay, J.F., Madupu, R., Daugherty, S., Brinkac, L., Beanan, M.J., Haft, D.H., Nelson, W.C., Davidsen, T., Zafar, N., Zhou, L., Liu, J., Yuan, Q., Khouri, H., Fedorova, N., Tran, B., Russell, D., Berry, K., Utterback, T., Van Aken, S.E., Feldblyum, T.V., D'Ascenzo, M., Deng, W-L., Ramos, A.R., Alfano, J.R., Cartinhour, S., Chatterjee, A.K., Delaney, T.P., Lazarowitz, S.G., Martin, G.B., Schneider, D.J., Tang, X., Bender, C.L., White, O., Fraser, C.M. and Collmer, A. (2003) The complete genome sequence of the Arabidopsis and tomato pathogen *Pseudomonas syringae* pv. *tomato* DC3000. *Proc. Natl. Acad. Sci. USA*, **100**, 10 181–10 186.

Charkowski, A.O., Alfano, J.R., Preston, G., Yuan, J., He, S.Y. and Collmer, A. (1998) The *Pseudomonas syringae* pv. *tomato* HrpW protein has domains similar to harpins and pectate lyases and can elicit the plant hypersensitive response and bind to pectate. *J. Bacteriol.* **180**, 5211–5217.

Chisholm, S.T., Coaker, G., Day, B. and Staskawicz, B.J. (2006) Host–microbe interactions: shaping the evolution of the plant immune response. *Cell*, **124**, 803–814.

Clarke, C.R., Chinchilla, D., Hind, S.R., Taguchi, F., Miki, R., Ichinose, Y., Martin, G.B., Leman, S., Felix, G. and Vinatzer, B.A. (2013) Allelic variation in two distinct *Pseudomonas syringae* flagellin epitopes modulates the strength of plant immune responses but not bacterial motility. *New Phytol.* **200**, 847–860.

Cunnac, S., Chakravarthy, S., Kvitko, B.H., Russell, A.B., Martin, G.B. and Collmer, A. (2011) Genetic disassembly and combinatorial reassembly identify a minimal functional repertoire of type III effectors in *Pseudomonas syringae*. *Proc. Natl. Acad. Sci. USA*, **108**, 2975–2980.

Dangl, J.L., Horvath, D.M. and Staskawicz, B.J. (2013) Pivoting the plant immune system from dissection to deployment. *Science*, **341**, 746–751.

Daunay, M.-C. (2008) Eggplant. In: *Vegetables II* (Prohens, J. and Nuez, F., eds), pp. 163–220. New York: Springer.

Doganlar, S., Frary, A., Daunay, M.-C., Lester, R.N. and Tanksley, S.D. (2002) A comparative genetic linkage map of eggplant (*Solanum melongena*) and its implications for genome evolution in the Solanaceae. *Genetics*, **161**, 1697–1711.

Elliott, C. (1951) *Manual of Bacterial Plant Pathogens*, Vol. 2. Waltham, MA: Chronica Botanica Company.

Felix, G., Duran, J.D., Volko, S. and Boller, T. (1999) Plants have a sensitive perception system for the most conserved domain of bacterial flagellin. *Plant J.* **18**, 265–276.

Feng, F. and Zhou, J.-M. (2012) Plant–bacterial pathogen interactions mediated by type III effectors. *Curr. Opin. Plant Biol.* **15**, 469–476.

Flor, H.H. (1971) Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* **9**, 275–296.

Gassmann, W., Dahlbeck, D., Chesnokova, O., Minsavage, G.V., Jones, J.B. and Staskawicz, B.J. (2000) Molecular evolution of virulence in natural field strains of *Xanthomonas campestris* pv. *vesicatoria*. *J. Bacteriol.* **182**, 7053–7059.

Gimenez-Ibanez, S., Hann, D.R., Ntoukakis, V., Petutschnig, E., Lipka, V. and Rathjen, J.P. (2009) AvrPtoB targets the LysM receptor kinase CERK1 to promote bacterial virulence on plants. *Curr. Biol.* **19**, 423–429.

Göhre, V., Spallek, T., Häweker, H., Mersmann, S., Mentzel, T., Boller, T., de Torres, M., Mansfield, J.W. and Robatzek, S. (2008) Plant pattern-recognition receptor FLS2 is directed for degradation by the bacterial ubiquitin ligase AvrPtoB. *Curr. Biol.* **18**, 1824–1832.

Grant, S.R., Fisher, E.J., Chang, J.H., Mole, B.M. and Dangl, J.L. (2006) Subterfuge and manipulation: type III effector proteins of phytopathogenic bacteria. *Annu. Rev. Microbiol.* **60**, 425–449.

Halpin, C. and Douglas, E. (2010) Gene stacking. In: *Molecular Techniques in Crop Improvement*, 2nd edn (Jain, S.M. and Brar, D.S., eds), pp. 613–629. Dordrecht, Heidelberg, London, New York: Springer.

Hirano, S.S., Charkowski, A.O., Collmer, A., Willis, D.K. and Upper, C.D. (1999) Role of the Hrp type III protein secretion system in growth of *Pseudomonas syringae* pv. *syringae* B728a on host plants in the field. *Proc. Natl. Acad. Sci. USA*, **96**, 9851–9856.

Jones, J.B., Lacy, G.H., Bouzar, H., Stall, R.E. and Schaad, N.W. (2004) Reclassification of the xanthomonads associated with bacterial spot disease of tomato and pepper. *Syst. Appl. Microbiol.* **27**, 755–762.

Katagiri, F. and Tsuda, K. (2010) Understanding the plant immune system. *Mol. Plant–Microbe Interact.* **23**, 1531–1536.

Kim, M.G., da Cunha, L., McFall, A.J., Belkhadir, Y., DebRoy, S., Dangl, J.L. and Mackey, D. (2005) Two *Pseudomonas syringae* type III effectors inhibit RIN4-regulated basal defense in arabidopsis. *Cell*, **121**, 749–759.

Kim, Y.J., Lin, N.-C. and Martin, G.B. (2002) Two distinct *Pseudomonas* effector proteins interact with the Pto kinase and activate plant immunity. *Cell*, **109**, 589–598.

Kunkeaw, S., Tan, S. and Coaker, G. (2010) Molecular and evolutionary analyses of *Pseudomonas syringae* pv. *tomato* Race 1. *Mol. Plant–Microbe Interact.* **23**, 415–424.

Kvitko, B.H., Park, D.H., Velasquez, A.C., Wei, C.-F., Russell, A.B., Martin, G.B., Schneider, D.J. and Collmer, A. (2009) Deletions in the repertoire of *Pseudomonas syringae* pv. *tomato* DC3000 type III secretion effector genes reveal functional overlap among effectors. *PLoS Pathog.* **5**, e1000388.

Lacombe, S., Rougon-Cardoso, A., Sherwood, E., Peeters, N., Dahlbeck, D., van Esse, H.P., Smoker, M., Rallapalli, G., Thomma, B.P.H.J, Staskawicz, B., Jones, J.D.G. and Zipfel, C. (2010) Interfamily transfer of a plant pattern-recognition receptor confers broad-spectrum bacterial resistance. *Nat. Biotechnol.* **28**, 365–369.

Lee, J., Klessig, D.F. and Nürnberger, T. (2001) A harpin binding site in tobacco plasma membranes mediates activation of the pathogenesis-related gene HIN1 independent of extracellular calcium but dependent on mitogen-activated protein kinase activity. *Plant Cell Online*, **13**, 1079–1093.

Martin, G.B. (2012) Suppression and activation of the plant immune system by *Pseudomonas syringae* effectors AvrPto and AvrPtoB. In: *Effectors in Plant–Microbe Interactions* (Martin, F. and Kamoun, S., eds), pp. 123–154. Ames: Wiley-Blackwell.

Martin, G.B., Brommonschenkel, S.H., Chunwongse, J., Frary, A., Ganal, M.W., Spivey, R., Wu, T., Earle, E.D. and Tanksley, S.D. (1993) Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science*, **262**, 1432–1436.

Monaghan, J. and Zipfel, C. (2012) Plant pattern recognition receptor complexes at the plasma membrane. *Curr. Opin. Plant Biol.* **15**, 349–357.

Noël, L., Thieme, F., Nennstiel, D. and Bonas, U. (2001) cDNA-AFLP analysis unravels a genome-wide hrpG-regulon in the plant pathogen *Xanthomonas campestris* pv. *vesicatoria*. *Mol. Microbiol.* **41**, 1271–1281.

Nomura, K., Melotto, M. and He, S.-Y. (2005) Suppression of host defense in compatible plant–*Pseudomonas syringae* interactions. *Curr. Opin. Plant Biol.* **8**, 361–368.

Oh, H.-S., Park, D.H. and Collmer, A. (2010) Components of the *Pseudomonas syringae* type III secretion system can suppress and may elicit plant innate immunity. *Mol. Plant–Microbe Interact.* **23**, 727–739.

Pitblado, R.E., MacNeill, B.H. and Kerr, E.A. (1984) Chromosomal identity and linkage relationships of Pto, a gene for resistance to *Pseudomonas syringae* pv. *tomato* in tomato. *Can. J. Plant Pathol.* **6**, 48–53.

Tai, T.H., Dahlbeck, D., Clark, E.T., Gajiwala, P., Pasion, R., Whalen, M.C., Stall, R.E. and Staskawicz, B.J. (1999) Expression of the Bs2 pepper gene confers resistance to bacterial spot disease in tomato. *Proc. Natl. Acad. Sci. USA*, **96**, 14 153–14 158.

Thomas, W.J., Thireault, C.A., Kimbrel, J.A. and Chang, J.H. (2009) Recombineering and stable integration of the *Pseudomonas syringae* pv. *syringae* 61 hrp/hrc cluster into the genome of the soil bacterium *Pseudomonas fluorescens* Pf0-1. *Plant J.* **60**, 919–928.

Wu, F., Eannetta, N., Xu, Y. and Tanksley, S. (2009) A detailed synteny map of the eggplant genome based on conserved ortholog set II (COSII) markers. *Theor. Appl. Genet.* **118**, 927–935.

Yan, S., Liu, H., Mohr, T.J., Jenrette, J., Chiodini, R., Zaccardelli, M., Setubal, J.C. and Vinatzer, B.A. (2008) Role of recombination in the evolution of the model plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000, a very atypical tomato strain. *Appl. Environ. Microbiol.* **74**, 3171–3181.

Zeng, L., Velásquez, A.C., Munkvold, K.R., Zhang, J. and Martin, G.B. (2012) A tomato LysM receptor-like kinase promotes immunity and its kinase activity is inhibited by AvrPtoB. *Plant J.* **69**, 92–103.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Fig. S1 Disease symptoms elicited by *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pto*DC3000) following spray infection [optical density at 600 nm (OD_{600}) = 0.01] of eggplant accessions MM738 (A) and MM195 (B). Photographs were taken 5 days following spray inoculation. Inset image shows a magnified view of the observed speck symptoms.

Fig. S2 Representative photographs of rapid leaf collapse elicited by *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pto*DC3000) wild-type and mutants on accessions from Table 2: 1, wild-type; 2, Δ*avrpto1*Δ*avrptoB*; 3, D28E; 4, D28E+*avrptoB*; 5, Δ*hrcC*. Photographs were taken 24 h after infiltration at an optical density at 600 nm (OD_{600}) = 0.1. All infiltration areas considered to show significant cell death are indicated with an asterisk.

Fig. S3 The rapid cell death elicited by *Pseudomonas syringae* pv. *tomato* strain DC3000D28E (*Pto*DC3000D28E) and *Pseudomonas fluorescens* (*Pf*) 0-1 EtHAN is present and different between eggplant accessions 48 and 72 h following inoculation. (A, B) Representative photographs and assigned leaf collapse scores of the macroscopic cell death triggered by *Pto*DC3000 and mutant strains (strain numbers listed above B) on MM134 and MM738, 2 days following infiltration at an optical density at 600 nm (OD_{600}) = 0.1 and 0.01. In a subset of experimental replicates on MM738, *Pto*DC3000D28E only elicited marginal cell death (Table 2 for example), but we do not group it with MM134 because, in other experiments, *Pto*DC3000D28E elicited significant cell death on MM738 (here, for example). (C, D) Representative photographs and assigned leaf collapse scores of the macroscopic cell death triggered by *Pf* 0-1 wild-type and *Pf* 0-1 EtHAN on MM134 and MM738, 3 days following infiltration at OD_{600} = 0.1 and 0.01. All infiltration areas considered to show significant cell death are indicated with an asterisk. Similar results were obtained in at least two independent experiments for all sections.

Fig. S4 Representative photographs of macroscopic cell death elicited by *Pseudomonas fluorescens* (*Pf*) 0-1 wild-type (strain 1) and EtHAN (strain 2) on the same accessions as in Fig. 2. Photographs were taken 24 h after infiltration at an optical density at 600 nm (OD_{600}) = 0.1. All infiltration areas considered to show significant cell death are indicated with an asterisk.

Fig. S5 Expression of the *Pseudomonas syringae* type III secretion system in *Pseudomonas fluorescens* (*Pf*) 0-1 elicits macroscopic cell death in a subset of Solanaceae. Leaves were infiltrated with either *Pf* 0-1 wild-type (right) or *Pf* 0-1 expressing the *P. syringae* T3SS (EtHAN, left). Bacteria were infiltrated at an optical density at 600 nm (OD_{600}) = 0.1 for tomato (*Solanum lycopersicum* cv. Rio Grande), tobacco (*Nicotiana tabacum* cv. Burly) and *N. benthamiana*, or OD_{600} = 0.3 for Arabidopsis (*A. thaliana* eco. Col-0). Photographs were taken 24 h after infiltration. All infiltration areas considered to show significant cell death are indicated with an asterisk. Essentially identical results were obtained in at least two independent experiments.

Fig. S6 *Pseudomonas syringae* pv. *tomato* strain DC3000D28E (*Pto*DC3000D28E) does not elicit macroscopic leaf collapse on tobacco (*Nicotiana tabacum* cv. Burly) or *N. benthamiana*. Strains (1, wild-type; 2, Δ*avrpto1*Δ*avrptoB*; 3, D28E; 4, D28E+*avrptoB*; 5, Δ*hrcC*) were infiltrated at an optical density at 600 nm (OD_{600}) = 0.1. Symptoms were scored (0–3 scoring system) and photographs were taken 24 h after infiltration. All infiltration areas considered to show significant cell death are indicated with an asterisk. Similar results were obtained in three independent experiments.

Fig. S7 Representative photographs of disease symptoms elicited by *Xanthomonas euvesicatoria* (*Xeu*) on accessions from Fig. 4. Photographs were taken 8 days following *Xeu* infiltration at an optical density at 600 nm (OD_{600}) = 0.001.

Fig. S8 *Xanthomonas euvesicatoria* (*Xeu*) does not trigger macroscopic leaf collapse on any of the tested accessions. The lack of hypersensitive response (HR)-like symptoms was observed 48 h after inoculation at an optical density at 600 nm (OD_{600}) = 0.1 on five separate leaves for each accession in two independent experiments.

Table S1 Statistical comparison of wild-type *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pto*DC3000) across the tested eggplant accessions.