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1 **Title**

2 Roq1 confers resistance to *Xanthomonas*, *Pseudomonas* syringae and *Ralstonia solanacearum*
3 in tomato

4

5 **Authors**

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17

[1] N.C.T. and A.S. wrote the manuscript and performed *Pseudomonas* and *Ralstonia* petiole infection assays. A.S. carried out *Xanthomonas* infection and *Agrobacterium* transient expression experiments. U.S.G. and S.F.H. performed *Xanthomonas* field experiments. C.G.H. constructed the *Ralstonia* knockout and performed *Ralstonia* soil soak assays, supervised by C.A. All authors analyzed results, edited and approved the manuscript.

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18

19 **Summary**

20 A single immune receptor expressed in tomato confers strong resistance to three
21 different bacterial diseases.

22

23 **Abstract**

24 *Xanthomonas* species, *Pseudomonas syringae* and *Ralstonia solanacearum* are bacterial plant
25 pathogens that cause significant yield loss in many crop species. Current control methods for
26 these pathogens are insufficient but there is significant potential for generating new disease-
27 resistant crop varieties. Plant immune receptors encoded by nucleotide-binding, leucine-rich
28 repeat (NLR) genes typically confer resistance to pathogens that produce a cognate elicitor,
29 often an effector protein secreted by the pathogen to promote virulence. The diverse sequence
30 and presence / absence variation of pathogen effector proteins within and between pathogen
31 species usually limits the utility of a single NLR gene to protecting a plant from a single
32 pathogen species or particular strains. The NLR protein Recognition of XopQ 1 (Roq1) was
33 recently identified from the plant *Nicotiana benthamiana* and mediates perception of the effector
34 proteins XopQ and HopQ1 from *Xanthomonas* and *P. syringae* respectively. Unlike most
35 recognized effectors, alleles of XopQ/HopQ1 are highly conserved and present in most plant
36 pathogenic strains of *Xanthomonas* and *P. syringae*. A homolog of XopQ/HopQ1, named RipB,
37 is present in many *R. solanacearum* strains. We found that Roq1 also mediates perception of
38 RipB and confers immunity to *Xanthomonas*, *P. syringae*, and *R. solanacearum* when
39 expressed in tomato. Strong resistance to *Xanthomonas perforans* was observed in three
40 seasons of field trials with both natural and artificial inoculation. The *Roq1* gene can therefore
41 be used to provide safe, economical and effective control of these pathogens in tomato and
42 other crop species and reduce or eliminate the need for traditional chemical controls.

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43

44 Main

45 Bacterial pathogens from the species *Pseudomonas syringae*, *Ralstonia solanacearum,* and the
46 genus *Xanthomonas* can infect many different crop species and inflict significant yield losses
47 when environmental conditions favor disease. *Xanthomonas* and *P. syringae* tend to enter plant
48 stem, leaf, or flower tissue through wounds or natural openings, such as stomata or
49 hydathodes, whereas *R. solanacearum* is soilborne, entering roots through wounds and natural
50 openings before colonizing xylem tissue (Vasse, Frey, and Trigalet 1995; Gudesblat, Torres,
51 and Vojnov 2009). Once inside the host these bacteria manipulate host metabolism and
52 suppress plant immunity using multiple strategies, including effector proteins delivered by the
53 type III secretion system (Kay and Bonas 2009; Peeters et al. 2013; Xin, Kvitko, and He 2018).
54 This enables the pathogens to multiply to high titers while the plant tissue is still alive and
55 showing few or no visual symptoms. Once the bacteria reach high populations they typically
56 cause necrosis of infected leaf tissue or wilting and eventual death of the plant.
57 Effective control measures for bacterial pathogens are relatively limited, particularly once plants
58 become infected (Davis et al. 2013). Soil fumigation can reduce *R. solanacearum* populations in
59 the soil but this is expensive, potentially hazardous to workers and the environment, and of
60 limited efficacy (Yuliar, Nion, and Toyota 2015). Copper sulfate and antibiotics such as
61 streptomycin have been used to control *Xanthomonas* species and *P. syringae* but have
62 adverse environmental impacts and many strains have evolved tolerance to these chemicals
63 (Kennelly et al. 2007; Griffin et al. 2017). Applying chemicals that induce systemic acquired
64 resistance, such as acibenzolar-S-methyl, can provide partial control but increase production
65 cost and can depress crop yields when used repeatedly (Pontes et al. 2016).
66 The most effective, economical, and safe way to control bacterial pathogens is to plant crop
67 varieties that are immune to the target pathogen (Jones et al. 2014; Vincelli 2016). Such

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68 immunity is often mediated by plant immune receptor genes. Plants have large families of cell
69 surface and intracellular immune receptor proteins that surveil for the presence of invading
70 pathogens (Zipfel 2014; Jones, Vance, and Dangl 2016). Effector proteins delivered by the
71 bacterial type III secretion system are common ligands for intracellular plant immune receptors
72 encoded by intracellular nucleotide-binding domain and leucine-rich repeat containing (NLR)
73 genes (X. Li, Kapos, and Zhang 2015; Jones, Vance, and Dangl 2016; Kapos, Devendrakumar,
74 and Li 2019). While effector proteins contribute to virulence on a susceptible host, an immune
75 response is activated in the plant if that plant has the cognate receptor to recognize the effector.
76 NLR genes typically confer strong, dominant resistance to pathogens that deliver the cognate
77 recognized effector protein (Jones and Dangl 2006; Boller and He 2009; Deslandes and Rivas
78 2012; X. Li, Kapos, and Zhang 2015). Disease-resistant plants can be generated by identifying
79 the appropriate plant immune receptor genes and transferring them into the target crop species
80 (Dangl, Horvath, and Staskawicz 2013).
81 We recently identified the *Nicotiana benthamiana* immune receptor gene named *Recognition of*
82 *XopQ 1* (*Roq1*), which appears to be restricted to the genus *Nicotiana* and is required for
83 resistance to *Xanthomonas spp.* and *P. syringae* (Schultink et al. 2017). *Roq1* is a
84 Toll/Interleukin-1 Receptor (TIR) NLR immune receptor that mediates recognition of the
85 *Xanthomonas* effector protein XopQ and the homologous effector HopQ1 from *P. syringae*.
86 XopQ is present in most species and strains of *Xanthomonas* (Ryan et al. 2011) and HopQ1 is
87 present in 62% (290 of 467) sequenced putative pathogenic *P. syringae* strains (Dillon et al.
88 2019). XopQ/HopQ1 has homology to nucleoside hydrolases and has been shown to enhance
89 virulence on susceptible hosts (Ferrante and Scortichini 2009; W. Li et al. 2013), possibly by
90 altering cytokinin levels or interfering with the activity of host 14-3-3 proteins (W. Li et al. 2013;
91 Giska et al. 2013; Teper et al. 2014; Hann et al. 2014). The conservation of XopQ/HopQ1 and
92 their importance in virulence suggests that *Roq1* has widespread potential to confer resistance
93 to these pathogens in diverse crop species. Indeed, transient expression assays demonstrated

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94 that *Roq1* can recognize XopQ/HopQ1 alleles from *Xanthomonas* and *P. syringae* pathogens of
95 tomato, pepper, rice, citrus, cassava, brassica, and bean (Schultink et al. 2017). However, it
96 was not known if *Roq1* can confer disease resistance when expressed in a crop plant.

97 Tomato is one of the most important vegetable crops and is highly susceptible to several
98 bacterial diseases. Bacterial spot, bacterial speck and bacterial wilt of tomato are caused by
99 *Xanthomonas* species, *P. syringae* pv. *tomato* and *R. solanacearum,* respectively. These
100 diseases are difficult to control, especially if the pathogens become established in a field and
101 environmental conditions favor disease (Rivard et al. 2012; Potnis et al. 2015). Tomato breeding
102 germplasm has only limited resistance against these diseases and in some cases linkage drag
103 has complicated introgression of resistance genes from wild relatives (Sharma and Bhattarai
104 2019). *R. solanacearum* contains a homolog of XopQ/HopQ1 called RipB, suggesting that
105 expressing *Roq1* in tomato could also confer resistance to bacterial wilt. Like XopQ/HopQ1 in
106 *Xanthomonas* and *P. syringae*, RipB is highly conserved in most *R. solanacearum* isolates
107 (Sabbagh et al. 2019). Here we present laboratory and field data showing that *Roq1* confers
108 resistance against these three pathogens in tomato and that this resistance depends on the
109 presence of the cognate pathogen effector.

110

111 Results

112 Tomatoes expressing *Roq1* are resistant to *Xanthomonas* and *P. syringae*

113 We generated homozygous tomato plants expressing the *Roq1* gene from *N. benthamiana* and
114 tested them for resistance to *Xanthomonas* and *P. syringae* by measuring bacterial growth *in*
115 *planta*. Population sizes of wild-type *X. perforans* strain 4B and *X. euvesicatoria* strain 85-10
116 were approximately 100-fold smaller in tomatoes expressing *Roq1* compared to wild-type
117 tomatoes at six days post inoculation (Fig. 1). In contrast, XopQ deletion mutants multiplied
118 equally well in leaves of both wild-type and *Roq1* tomato. Disease symptoms begin as small

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119 water-soaked lesions and progress to necrosis of infected tissue. Wild-type *X. perforans* and *X.*
120 *euvesicatoria* caused severe disease symptoms on wild-type tomato plants but failed to cause
121 visible symptoms on *Roq1* plants (Fig. 2). The XopQ mutants caused similar disease symptoms
122 on both wild-type and *Roq1* tomato. Similar results were observed for *P. syringae* DC3000 and
123 its HopQ1 mutant (Fig. 1, 2) and a Race 1 isolate of *P. syringae* pv. *tomato* (Supplementary Fig.
124 1).
125
126 **Expression of *Roq1* confers resistance to *Xanthomonas perforans* in the field**
127 To determine if the resistance observed in growth chamber experiments would hold up under
128 commercial tomato production conditions, we tested the ability of *Roq1* tomatoes to resist *X.*
129 *perforans* infection in the field. *Roq1* tomatoes were grown along with the Fla. 8000 wild-type
130 parent as well as a Fla. 8000 variety expressing the Bs2 gene from pepper as a resistant control
131 (Kunwar et al. 2018). For each of the three growing seasons both *Roq1* and the resistant *Bs2*
132 control tomatoes showed significantly lower disease severity than the parental Fla. 8000 variety
133 (Table 1) ($p < 0.05$). The total marketable yield of the *Roq1* plants was not significantly different
134 from that of the susceptible parent for any of the three seasons ($p > 0.05$).
135
136 **The *R. solanacearum* RipB effector, a homolog of XopQ/HopQ1, is recognized by Roq1**
137 RipB, considered a “core” effector of *R. solanacearum*, is present in approximately 90% of
138 sequenced strains (Sabbagh et al. 2019), making it an attractive target ligand for engineering
139 crop plants to be resistant to this pathogen. Roq1 perceives diverse alleles of XopQ and HopQ1
140 and we hypothesized that it can also recognize RipB. We constructed a phylogenetic tree of
141 RipB, XopQ, and HopQ1 alleles identified by BLAST search and observed two major clades of
142 RipB proteins, corresponding to phylotypes I and III (strains originating in Asia and Africa) and
143 to phylotype II (strains originating in the Americas) (Fig. 3). We selected RipB alleles from *R.*
144 *solanacearum* strains GMI1000 and MolK2, which are present in clades 1 and 2, respectively,

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145 for subsequent analysis. These two RipB alleles share 71% amino acid identity with each other
146 and approximately 52% identity with XopQ excluding the divergent N terminus containing the
147 putative type III secretion signal. An alignment of these two RipB proteins with XopQ and
148 HopQ1 is shown in Supplementary Fig. 2. To test for Roq1-dependent recognition of RipB, we
149 used *Agrobacterium* to transiently express RipB from GMI1000 and Molk2 in leaf tissue of wild-
150 type and *roq1* mutant *N. tabacum*. Both RipB alleles triggered a strong hypersensitive / cell
151 death response in wild-type *N. tabacum,* indicating immune activation. This response was
152 absent in the *roq1-1* mutant but could be restored by transiently expressing Roq1 along with
153 XopQ, $\text{RipB}_{\text{GMI1000}}$, or $\text{RipB}_{\text{Molk2}}$ (Fig. 4).
154

155 ***Roq1* tomatoes are resistant to *R. solanacearum* containing RipB**

156 Our observation that Roq1 can recognize RipB in leaf transient expression assays suggested
157 that Roq1 can mediate resistance to bacterial wilt caused by *R. solanacearum*. We tested this
158 hypothesis by challenging wild-type and Roq1-expressing tomato plants with *R. solanacearum*
159 strain GMI1000 using a soil soak inoculation disease assay. Wild-type plants developed severe
160 wilting approximately seven days after inoculation, whereas *Roq1* tomato plants remained
161 mostly healthy over the two-week time course. The *Roq1* tomato plants were susceptible to a
162 deletion mutant lacking RipB (GMI1000 *ΔripB*) (Fig. 5a). We also challenged plants by
163 introducing bacteria directly to the xylem by placing bacteria on the surface of a cut petiole.
164 Wild-type plants were wilted at eight days whereas *Roq1* plants remained healthy (Fig. 5b).
165 Tomatoes expressing *Roq1* were also resistant to *R. solanacearum* strain UW551, which is a
166 Race 3 Biovar 2 potato brown rot strain that has a clade 2 RipB allele (Supplementary Fig. 3).
167

168 **Occurrence of RipB in the *R. solanacearum* species complex**

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169 To investigate the potential for using *Roq1* to protect plants from *R. solanacearum*, we
170 investigated the occurrence of RipB alleles in select *R. solanacearum* strains. Table 2
171 summarizes published known hosts of several *R. solanacearum* strains along with the phylotype
172 and identified RipB allele. All strains in Table 2 except for tobacco pathogenic strains K60, Y45,
173 BK1002 and OE1-1 contain putative full-length and functional RipB alleles. Relative to other
174 RipB alleles, the K60 RipB allele is truncated at residue 437 and missing approximately 65 C-
175 terminal residues and the OE1-1 allele is truncated at residue 425, missing approximately 77
176 residues based on a published genome sequence (Hayes, MacIntyre, and Allen 2017). Y45
177 does not have a predicted RipB allele based on a draft genome sequence (Z. Li et al. 2011).
178

179 **Discussion**

180 *Roq1* expression in tomato confers strong resistance to *X. perforans*, *X. euvesicatoria and P.*
181 *syringae* pv. *tomato*. Its effectiveness is dependent on the presence of the recognized effector
182 protein XopQ/HopQ1 (Fig. 1, 2). Field trials revealed that tomatoes expressing *Roq1* were less
183 susceptible to *X. perforans* than wild-type tomatoes in conditions approximating commercial
184 production (Table 1). *Roq1* conferred a similar level of resistance as the Bs2-containing
185 resistant check variety in one season and was slightly weaker in the other two. Bacterial spot
186 caused by *X. perforans* can cause lesions on fruits, making them unsuitable for commercial
187 sale, and also reduce plant productivity by damaging leaf tissue. Despite showing strong
188 disease resistance, the tested *Roq1* line did not give a significantly greater total marketable
189 yield than the susceptible parental variety. Of the three seasons, Spring 2019 had weather
190 conditions expected to be most conducive for observing an impact of bacterial spot on
191 marketable yield with mid-season rain promoting the early development of disease symptoms.
192 The average marketable yield for the *Roq1* tomatoes was 27% higher than wild-type in this
193 season, although a relatively small sample size (4 replicate plots of ten plants each) and a large

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194 variability of yield between plots resulted in a p-value of 0.08 by Student's t-test. Although we
195 cannot conclude that *Roq1* improves marketable yield of tomatoes from this data, a larger trial
196 under high disease pressure may reveal such a difference.

197 It was unclear if *Roq1* could confer resistance to *R. solanacearum* because it colonizes different
198 tissues than *Xanthomonas* and *P. syringae*. While *Xanthomonas* and *P. syringae* colonize
199 tomato leaf tissue, *R. solanacearum* enters through the roots and colonizes xylem vessels.
200 Although *R. solanacearum's* type III secretion system is essential for virulence, it is not clear
201 when and where the pathogen delivers effectors into host cells. It was therefore not clear if
202 *Roq1* would be able to confer resistance to this pathogen in tomato. Here we confirmed that
203 tomato plants expressing *Roq1* had strong resistance to *R. solanacearum* expressing RipB as
204 measured by both soil soak and cut-petiole inoculation assays. This result is consistent with and
205 expands on the recent report that RipB is recognized in *Nicotiana* species and that silencing
206 *Roq1* confers susceptibility to *R. solanacearum* in *Nicotiana benthamiana(Nakano and*
207 *Mukaihara 2019)*. In addition, *Roq1* confers resistance to *R. solanacearum* Race 3 biovar 3
208 strain UW551, a pathogen that can overcome other known sources of bacterial wilt resistance in
209 tomato(Milling, Babujee, and Allen 2011). Some but not all of the *Roq1* tomatoes inoculated by
210 the soil soak assay were colonized by *R. solanacearum* (Supplementary Fig. 4), implying that
211 Roq1 both restricts the establishment of vascular colonization and separately reduces bacterial
212 titers if colonization does occur. Activation of immune receptors, including Roq1, is known to
213 induce many defense-associated genes with different putative activities(Sohn et al. 2014; Qi et
214 al. 2018), presumably acting to inhibit pathogen virulence by distinct mechanisms. The
215 observation that Roq1 inhibits both colonization establishment and population growth suggest
216 that at least two independent downstream defense responses mediate the observed resistance
217 phenotype.

218 The *Roq1* tomatoes were fully susceptible to an *R. solanacearum* mutant lacking *RipB*,
219 indicating that the resistance depends on the interaction between RipB and Roq1. This is

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220 consistent with the observation that several naturally occurring *R. solanacearum* strains that can
221 infect tobacco have truncated or are missing the RipB effector (Table 2)(Nakano and Mukaihara
222 2019), suggesting that losing RipB can allow the pathogen to overcome the native *Roq1* gene
223 present in *Nicotiana tabacum*. Tobacco-infecting strains K60 and OE1-1 contain independently
224 truncated RipB alleles (Fig. 3) and there have likely been multiple independent gene loss events
225 which enable strains to evade Roq1-mediated resistance. Similarly, HopQ1 has been lost in
226 strains of *P. syringae* that can infect tobacco (Denny 2006; Ferrante and Scortichini 2009; Z. Li
227 et al. 2011). This suggests that this effector is not essential for virulence and it would therefore
228 be prudent to deploy *Roq1* in combination with other disease resistance traits to avoid
229 resistance breakdown due to pathogens losing XopQ/HopQ1/RipB.

230 No other known NLR immune receptor confers resistance against such a broad range of
231 bacterial pathogens as Roq1. Effectors that are recognized by NLR proteins act as avirulence
232 factors and are under strong evolutionary pressure to diversify or be lost to evade immune
233 activation. Therefore the effector repertoires of pathogens are often quite diverse, with relatively
234 few “core” effectors conserved within a species and even fewer shared between different
235 genera (Grant et al. 2006). Effectors recognized by plant NLRs are typically narrowly conserved
236 within a single bacterial genus (Kapos, Devendrakumar, and Li 2019). One such effector is
237 AvrBs2, recognized by the Bs2 receptor from pepper, which is present in many *Xanthomonas*
238 strains but is absent from *P. syringae* and *R. solanacearum*. In contrast XopQ/HopQ1/RipB is
239 highly conserved in most *Xanthomonas*, *P. syringae* and *R. solanacearum* strains that cause
240 disease in crop plants including kiwi (*P. syringae* pv. *actinidae*), banana (*R. solanacearum* and
241 *X. campestris* pv. *musacearum*), stone fruit (*P. syringae*), pepper (*X. euvesicatoria*), citrus (*X.*
242 *citri*), strawberry (*X. fragariae*), brassica (*X. campestris*), rice (*X. oryzae*), potato (*R.*
243 *solanacearum*) and others. *R. solanacearum* Race 3 Biovar 2 strains are of particular concern
244 because they are cold tolerant and potentially threaten potato cultivation in temperate climates.

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245 As a result, *R. solanacearum* Race 3 biovar 2 is a strictly regulated quarantine pathogen in
246 Europe and North America and is on the United States Select Agent list. The ability of *Roq1* to
247 protect tomato from the Race 3 Biovar 2 strain UW551 (Supplementary Fig. 3) suggests that
248 *Roq1* can also protect potato from this high-concern pathogen. This work demonstrates the
249 widespread potential of using naturally occurring plant immune receptors to manage diverse
250 and difficult to control pathogen species safely, sustainably and economically.
251

252 ## Methods

253 Generation of tomato expressing Roq1

254 The Roq1 coding sequence was amplified from *N. benthamiana* cDNA and cloned into the
255 pORE E4 binary plasmid (Coutu et al. 2007). *A. tumefaciens* co-cultivation was used to
256 transform *Roq1* into the commercial tomato variety Fla. 8000 at the University of Nebraska Plant
257 Transformation Core Research Facility. Transformed plants were selected by resistance to
258 kanamycin, confirmed by genotyping, and selfed to obtain homozygous lines.
259

260 Bacterial Leaf Spot and Leaf Speck disease assays

261 *Xanthomonas spp.* cultures were grown in NYG broth (0.5% peptone, 0.3% yeast extract, 2%
262 glycerol) with rifampicin (100 µg / mL) overnight at 30 °C. *P. syringae* cultures were grown in KB
263 broth (1% peptone, 0.15% K_2HPO_4, 1.5% glycerol, 5 mM $MgSO_4$, pH 7.0) with rifampicin (100
264 µg / mL) overnight at 28 °C. Bacterial cultures were spun down at 5200 *g*, washed once with 10
265 mM $MgCl_2$, and then diluted to the appropriate infiltration density with 10 mM $MgCl_2$. Leaf tissue
266 of tomato plants (approximately four weeks old) was infiltrated with bacterial solution using a
267 needleless syringe. To quantify bacterial growth, leaf punches were homogenized in water,
268 serially diluted and plated on NYG (for *Xanthomonas spp.*) or KB (for *P. syringae*) plates
269 supplemented with 100 µg / mL rifampicin and 50 µg / mL cycloheximide to measure colony

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270 forming units. *X. perforans* strain 4B, *X. euvesicatoria* strain 85-10, and *P. syringae* strain
271 DC3000 and the corresponding XopQ/HopQ1 deletion mutants were described previously
272 (Schwartz et al. 2015; Schultink et al. 2017). The *P. syringae* pv. *tomato* Race 1 strain was
273 isolated from a field of tomatoes with the PTO resistance gene in 1993 in California.
274
275 Transient expression of RipB and XopQ
276 Alleles of RipB from *R. solanacearum* (NCBI Genbank accessions CAD13773.2 and
277 WP_003278485) were synthesized and cloned into a BsaI-compatible version of the pORE E4
278 vector (Coutu et al. 2007). This plasmid was transformed into *A. tumefaciens* strain C58C1. *A.*
279 *tumefaciens* cultures were grown on a shaker overnight at 30 °C in LB broth with rifampicin (100
280 µg / mL), tetracycline (10 µg / mL) and kanamycin (50 µg / mL). The cells were collected by
281 centrifugation and resuspended in infiltration buffer (10 mM 2-(N-morpholino)ethanesulfonic
282 acid, 10 mM $MgCl_2$, pH 5.6), and diluted to an OD_{600} of 0.5 for infiltration into *N. tabacum* leaf
283 tissue.
284
285 *N. tabacum roq1* mutant lines
286 *N. tabacum roq1* mutant lines were generated by transforming *N. tabacum* with a construct
287 coding for CAS9 and a guide RNA targeting the Roq1 gene with the sequence
288 GATGATAAGGAGTTAAAGAG. This construct was also used for the generation of *N.*
289 *benthamiana roq1* mutants published in Qi et al. 2018. Transformed *N. tabacum* plants were
290 generated by Agrobacterium co-cultivation and selected for using kanamycin. Transformed
291 plants were genotyped for the presence of mutations at the target site by PCR and Sanger
292 sequencing. The *N. tabacum* mutant *roq1-1* has a single base pair A insertion at the target cut
293 site in the *Roq1* gene.
294
295 Bacterial wilt virulence assays

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296 *R. solanacearum* virulence on tomato was measured as previously described(Khokhani et al.
297 2018). Briefly, cells of *R. solanacearum* strain GMI1000 grown overnight in CPG (0.1% casein
298 hydrolysate, 1% peptone, 0.5% glucose, pH 7.0) at 28°C were collected by centrifugation and
299 diluted to an OD_{600} of 0.1 in water ($1x10^8$ CFU/ml). 50 mL of this suspension was poured on the
300 soil around 17-day old tomato plants. Disease was rated daily for two weeks on a 0-4 disease
301 index scale, where 0 is no leaves wilted, 1 is 1-25% wilted, 2 is 26-50% wilted, 3 is 51-75% of
302 wilted, and 4 is 76-100% wilted. Data represent a total of four biological replicates with ten
303 plants per replicate. Virulence data were analyzed using repeated measures ANOVA (Khokhani
304 et al 2018). For petiole infection, the petiole of the first true leaf was cut with a razor blade
305 horizontally approximately 1 cm from the stem. A drop of bacterial solution (2 uL, OD_{600} = 0.001)
306 was pipetted onto the exposed cut petiole surface.
307
308 Field trial disease assays
309 Three field trials were conducted at the University of Florida Gulf Coast Research and
310 Education Center in Balm during the spring seasons of 2018 and 2019 and the fall season of
311 2018 and under the notification process of the United States Department of Agriculture. Large-
312 fruited, fresh market tomato lines were used in these trials and included the inbred line, Fla.
313 8000, and nearly-isogenic lines containing either *Roq1* (event 316.4) or *Bs2* (Kunwar et al.
314 2018); along with commercial hybrids Florida 91, Sanibel, and HM 1823 as additional,
315 susceptible controls (data not shown). For each trial, seeds were sown directly into peat-lite
316 soilless media (Speedling, Sun City, FL, USA) in 128-cell trays (38 cm3 cell size). Transplants
317 were grown in a greenhouse until 5 or 6 weeks, then planted to field beds that had been
318 fumigated and covered with reflective plastic mulch. Field trials were conducted using a
319 randomized complete block design with four blocks and 10-plant plots. Field plants were staked
320 and tied, and irrigation was applied through drip tape beneath the plastic mulch of each bed. A
321 recommended fertilizer and pesticide program were followed throughout the growing season,

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322 excluding the use of plant defense inducers, copper, or other bactericides (Freeman et al.
323 2018). Fruit were harvested from the inner 8 plants of each plot at the breaker stage and
324 beyond graded for marketability according to USDA specifications. Yield data were analyzed
325 using the PROC GLIMMIX procedure in SAS (version 9.4; SAS Institute, Cary, NC, USA), and
326 block was considered random effects.

327 Field trials were inoculated with *X. perforans* race T4 (strain mixture of GEV904, GEV917,
328 GEV1001, and GEV1063). Bacterial strains were grown on nutrient agar medium (BBL, Becton
329 Dickinson and Co., Cockeysville, MD) and incubated at 28 °C for 24 h. Bacterial cells were
330 removed from the plates and suspended in a 10 mM $MgSo_4$·7H2O solution, and the suspension
331 was adjusted to OD_{600}=0.3, which corresponds to 10^8 CFU/ml. The suspension for each strain
332 was then diluted to 10^6 CFU/ml, mixed in equal volume, and applied along with polyoxyethylene
333 sorbitan monolaurate (Tween 20; 0.05% [vol/vol]) for field inoculation. Field trial plants were
334 inoculated approximately 3 weeks after transplanting.

335 Bacterial spot disease severity was recorded three to eight weeks after inoculation using the
336 Horsfall-Barratt scale (Horsfall, JG and Barrat, RW 1945), and ratings were converted to
337 midpoint percentages for statistical analysis. Disease severity data were analyzed using a
338 nonparametric procedure for the analysis of ordinal data (Brunner and Puri 2001; Shah and
339 Madden 2004). Analysis of variance type statistic of ranked data was conducted using the
340 PROC MIXED procedure in SAS. Relative marginal effects (RME) were generated with the
341 equation: RME = $(R – 0.5)/N$; where R is the mean treatment ranking, and N is the total number
342 of experimental units in the analysis; the LD_CI macro was used to generate 95% confidence
343 intervals (Brunner and Puri 2001; Shah and Madden 2004). Blocks were considered random
344 effects.

345 Generation of the *R. solanacearum ΔripB* mutant

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346 An unmarked *ΔripB* mutant was created using *sacB* positive selection with the vector pUFR80
347 (Castañeda et al. 2005). Briefly, the regions upstream and downstream of *ripB* were amplified
348 using the primers ripBupF/R and ripBdwnF/R. These fragments were inserted into pUFR80
349 digested with HindIII and EcoRI using Gibson Assembly (Gibson et al. 2009) (New England
350 Biolabs, Ipswitch, MA) and this construct was incorporated into the genome of strain GMI1000
351 using natural transformation, with successful integrants selected on CPG + kanamycin (Coupat
352 et al. 2008). Plasmid loss was then selected for on CPG plates containing 5% w/v sucrose.
353 Correct deletions were confirmed using PCR and sequencing.
354 Phylogenetic analysis of XopQ, HopQ1 and RipB alleles
355 RipB alleles were identified by BLAST search of the NCBI protein database. Clustal Omega
356 (Sievers et al. 2011) was used to generate a multiple sequence alignment with XopQ and
357 HopQ1 alleles. To span the diversity of RipB alleles without have many redundant sequences,
358 only a single sequence was retained if there were multiple identical or nearly identical
359 sequences identified. A maximum likelihood tree was generated using PhyML (Guindon et al.
360 2010).
361
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366 *tabacum*.
367
368 **Competing Interests**

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369 A. S. and N.C.T. are employees of and have a financial stake in Fortiphyte Inc., which has
370 intellectual property rights related to the Roq1 resistance gene.
371

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372 **Tables**

Season / Genotype	Disease severity	Marketable yield (kg/ha)
Spring 2018		
Fla. 8000	86 ± 5	54,655 ± 9,450
Fla. 8000 Roq1	1 ± 1	52,656 ± 3,810
Fla. 8000 Bs2	1 ± 1	66,270 ± 10,309
Fall 2018		
Fla. 8000	25 ± 7	19,576 ± 11,038
Fla. 8000 Roq1	5 ± 1	18,538 ± 5,901
Fla. 8000 Bs2	0 ± 0	33,770 ± 13,176
Spring 2019		
Fla. 8000	84 ± 2	73,009 ± 15,243
Fla. 8000 Roq1	11 ± 7	92,837 ± 11,072
Fla. 8000 Bs2	5 ± 1	80,516 ± 14,531

373

374 **Table 1.** Field trial results. A field trial was conducted in Florida with disease pressure from
375 *Xanthomonas perforans*. Disease severity was scored out of 100 using the Horsfall-Barratt
376 scale. Harvested tomatoes were graded and sized by USDA specifications to calculate the total
377 marketable yield. The values shown are means ± standard deviation from at least four replicate
378 plots of ten plants each. Tomato plants expressing the Bs2 immune receptor gene were
379 included as a resistant control.

Strain	Host(s)	Phylotype	RipB allele	RipB accession
GMI1000	Tomato, Pepper, Arabidopsis	I	Present	WP_011000212
RS1000	Tomato	I	Present	BAD42390
OE1-1	Tobacco	I	Truncated	APC67368
BK1002	Tobacco		Truncated	LC459955
Y45	Tobacco	IB	Absent	
K60	Tomato, Tobacco	IIA	Truncated	CCF97494
CFBP2957	Tomato	IIA	Present	CBJ44424
Po82	Tomato, Banana, Potato	IIB	Present	WP_014618158
IPO1609*	Potato	IIB	Present	WP_020956993
MolK2	Banana	IIB	Present	WP_003278485
UW551*	Geranium, Tomato	IIB	Present	EAP72492
CMR15	Tomato	III	Present	WP_020749919
PSI07	Tomato	IV	Present	WP_013213770
BDB R229	Banana		Present	WP_078222314

380

381 **Table 2.** RipB occurrence and host range in *Ralstonia solanacearum*. The published host range
382 of select *Ralstonia solanacearum* strains is listed along with the identified RipB allele. Truncated

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383 indicates that the identified allele is missing conserved residues at the C terminus and is
384 putatively non-functional. * indicates a Race 3 Biovar 2 Select Agent strain.

385 **Figure Legends**

386 **Figure 1.** Bacterial growth in tomatoes expressing Roq1*. Xanthomonas perforans* 4B (*Xp*),
387 *Xanthomonas euvesicatoria* 85-10 (*Xe*), and *Pseudomonas syringae* DC3000 (*Ps*) were
388 infiltrated into leaf tissue of wild-type tomato and tomato expressing Roq1 at a low inoculum
389 (OD_{600} = 0.0001 for Xe and Xp; OD_{600} = 0.00005 for Ps). Bacterial abundance was quantified by
390 homogenizing leaf punches and counting colony forming units (CFU) per square centimeter of
391 leaf tissue at six days post infiltration for *Xe* and *Xp;* three days post infiltration for *Ps*. Error bars
392 indicate standard deviation. * = $p < 0.05$, ** = $p < 0.01$ by Student's t-test.
393

394 **Figure 2.** Bacterial disease symptoms on *Roq1* tomato*. Xanthomonas perforans* 4B (*Xp*),
395 *Xanthomonas euvesicatoria* 85-10 (*Xe*), and *Pseudomonas syringae* DC3000 (*Ps*) wild-type and
396 XopQ/HopQ1 knockout strains were infiltrated into tomato leaf tissue at low inoculum and
397 disease symptoms were imaged at twelve, thirteen and four days post infiltration for *Xe*, *Xp* and
398 *Ps* respectively. The infiltration was performed using a needless syringe and circular wounds
399 from the infiltration are visible. The distal part of region of each leaf was infiltrated and the
400 proximal part was left untreated. *Xe* and *Xp* were infiltrated at an OD_{600} of 0.0001 whereas *Ps*
401 was infiltrated at an OD_{600} of 0.00005.

402 **Figure 3.** Phylogenetic tree of RipB proteins. Two major clades of RipB alleles from *Ralstonia*
403 *solanacearum* strains are visible in a maximum likelihood tree generated from XopQ, HopQ1
404 and RipB protein sequences. RipB alleles from *Ralstonia solanacearum* strains GMI1000 and
405 MolK2 were cloned for testing in this study and are indicated by pink dots. Several alleles have
406 truncations that may make them nonfunctional (indicated by a half circle). Abbreviations were

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407 used for *Ralstonia* (R) *and Ralstonia solanacearum* (Rs).

408 **Figure 4**. Roq1-dependent recognition of RipB in *Nicotiana tabacum*. *Agrobacterium*
409 *tumefaciens* was used to transiently expressed XopQ, $RipB_{GMI1000}$ and $RipB_{MolK2}$ along with
410 either Roq1 or an empty vector (EV) control in wild-type *Nicotiana tabacum* and a *roq1* loss of
411 function mutant. The *Agrobacterium* was infiltrated at a total OD_{600} of 0.5 and the leaves were
412 imaged at three days post infiltration.

413 **Figure 5.** Bacterial Wilt disease development in Roq1 tomatoes. (a) Wild-type and Roq1
414 tomatoes were infected with wild-type and RipB mutant *Ralstonia solanacearum* strain GMI1000
415 by soil soak inoculation. Disease symptoms were monitored over 14 days, with no wilting
416 corresponding to a Disease Index of 0 and complete wilting corresponding to a Disease Index of
417 4. Error bars indicate standard error. (b) Wild-type and Roq1 tomato plants 8 days after petiole
418 inoculation with approximately 2,000 cells of wild-type GMI1000.

419

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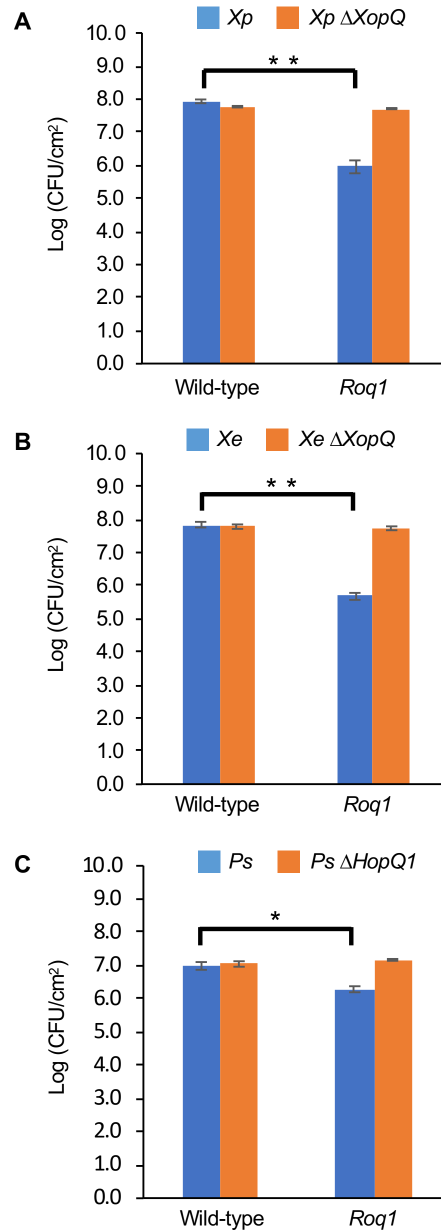

Figure 1. Bacterial growth in tomatoes expressing Roq1. *Xanthomonas perforans* 4B (*Xp*), *Xanthomonas euvesicatoria* 85-10 (*Xe*), and *Pseudomonas syringae* DC3000 (*Ps*) were infiltrated into leaf tissue of wild-type tomato and tomato expressing Roq1 at a low inoculum (OD_{600} = 0.0001 for Xe and Xp; OD_{600} = 0.00005 for Ps). Bacterial abundance was quantified by homogenizing leaf punches and counting colony forming units (CFU) per square centimeter of leaf tissue at six days post infiltration for *Xe* and *Xp;* three days post infiltration for *Ps*. Error bars indicate standard deviation. * = $p < 0.05$, ** = $p < 0.01$ by Student's t-test.

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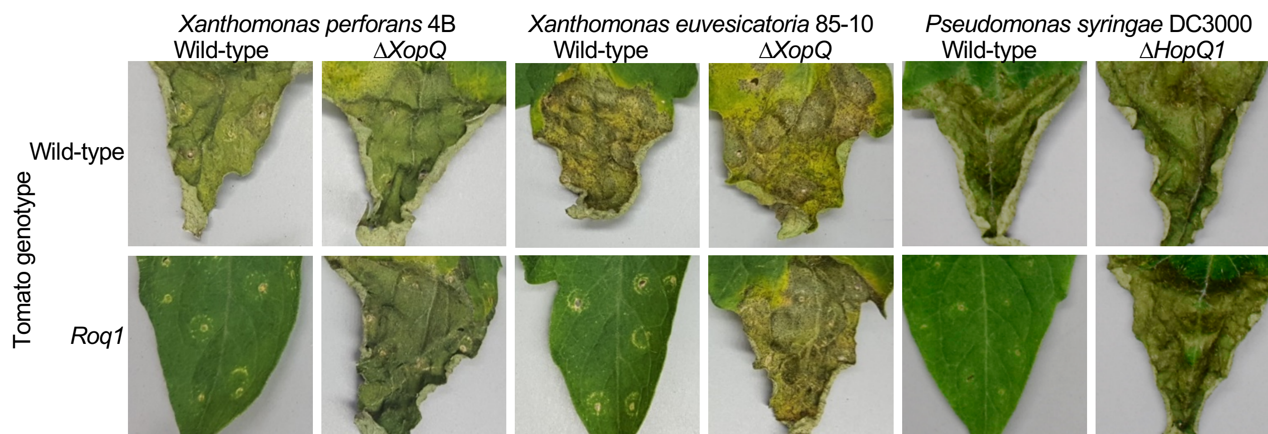

Figure 2. Bacterial disease symptoms on *Roq1* tomato. *Xanthomonas perforans* 4B (*Xp*), *Xanthomonas euvesicatoria* 85-10 (*Xe*), and *Pseudomonas syringae* DC3000 (*Ps*) wild-type and XopQ/HopQ1 knockout strains were infiltrated into tomato leaf tissue at low inoculum and disease symptoms were imaged at twelve, thirteen and four days post infiltration for *Xe*, *Xp* and *Ps* respectively. The infiltration was performed using a needless syringe and circular wounds from the infiltration are visible. The distal part of region of each leaf was infiltrated and the proximal part was left untreated. *Xe* and *Xp* were infiltrated at an OD_{600} of 0.0001 whereas *Ps* was infiltrated at an OD_{600} of 0.00005.

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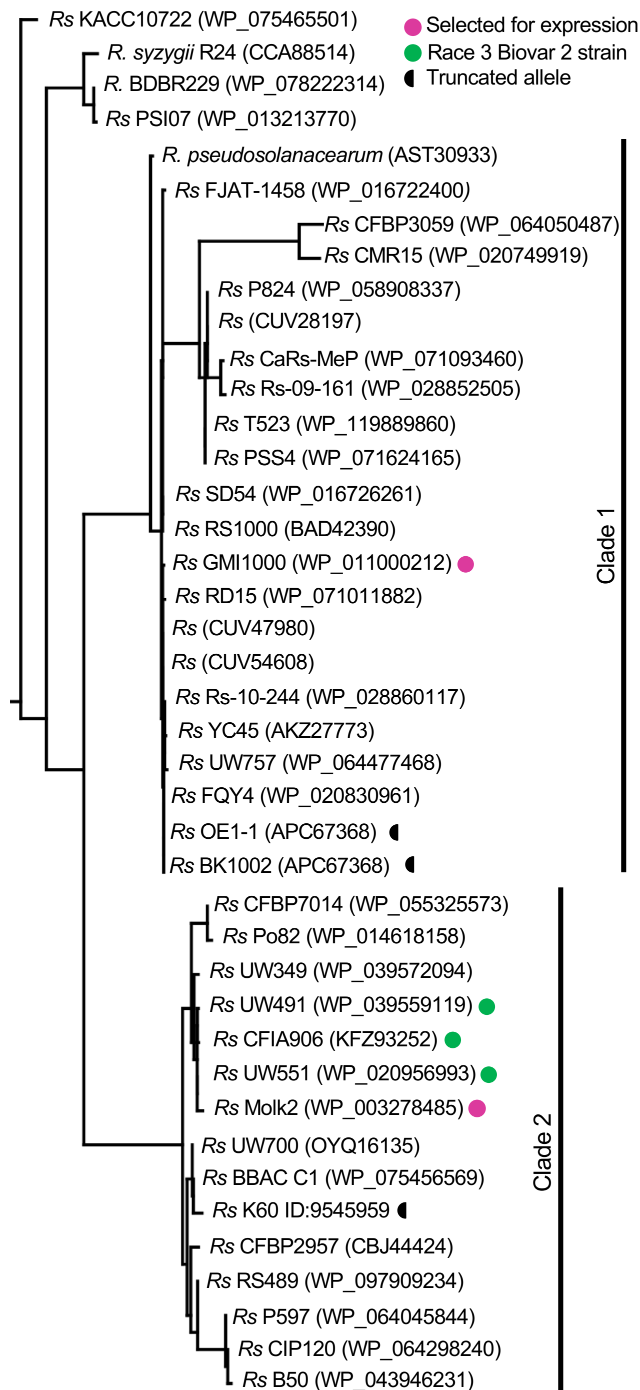

Figure 3. Phylogenetic tree of RipB proteins. Two major clades of RipB alleles from *Ralstonia solanacearum* strains are visible in a maximum likelihood tree generated from XopQ, HopQ1 and RipB protein sequences. RipB alleles from *Ralstonia solanacearum* strains GMI1000 and MolK2 were cloned for testing in this study and are indicated by pink dots. Several alleles have truncations that may make them nonfunctional (indicated by a half circle). Abbreviations were used for *Ralstonia* (R) *and Ralstonia solanacearum* (Rs).

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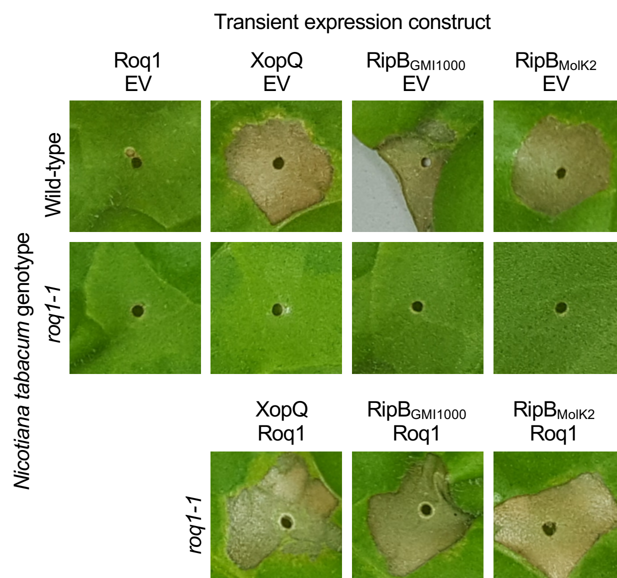

Figure 4.

Roq1-dependent recognition of RipB in *Nicotiana tabacum*. *Agrobacterium tumefaciens* was used to transiently expressed XopQ, RipB$_{GMI1000}$ and RipB$_{MolK2}$ along with either Roq1 or an empty vector (EV) control in wild-type *Nicotiana tabacum* and a *roq1* loss of function mutant. The *Agrobacterium* was infiltrated at a total OD_{600} of 0.5 and the leaves were imaged at three days post infiltration.

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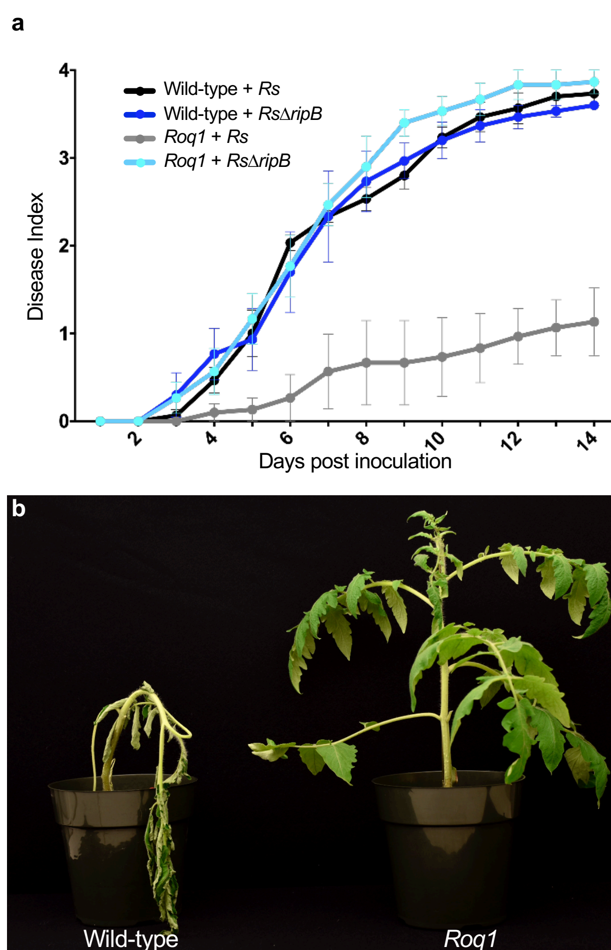

Figure 5.

Bacterial Wilt disease development in Roq1 tomatoes. (a) Wild-type and Roq1 tomatoes were infected with wild-type and RipB mutant *Ralstonia solanacearum* strain GMI1000 by soil soak inoculation. Disease symptoms were monitored over 14 days, with no wilting corresponding to a Disease Index of 0 and complete wilting corresponding to a Disease Index of 4. Error bars indicate standard error. (b) Wild-type and Roq1 tomato plants 8 days after petiole inoculation with approximately 2,000 cells of wild-type GMI1000.

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