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1 **Improving common bacterial blight phenotyping by using rub-**
2 **inoculation and machine learning: cheaper, better, faster, stronger**

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7

8 **Keywords:** phenotyping, plant disease, bacterial blight, *Xanthomonas*, TAL effectors, *Phaseolus*
9 *vulgaris*.

10

11 **ABSTRACT**

12 Accurate assessment of plant symptoms plays a key role for measuring the impact of pathogens
13 during plant-pathogen interaction. Common bacterial blight caused by *Xanthomonas phaseoli* pv.
14 *phaseoli* and *Xanthomonas citri* pv. *fuscans* (*Xpp-Xcf*) is a major threat to common bean. The
15 pathogenicity of these bacteria is variable among strains, and depends mainly on a type III secretion
16 system and associated type III effectors such as transcription activator-like effectors (TALEs).
17 Because the impact of a single gene is often small and difficult to detect, a discriminating
18 methodology is required to distinguish the slight phenotype changes induced during the progression
19 of the disease. Here, we compared two different inoculation and symptom assessment methods for
20 their ability to distinguish two *tal* mutants from their corresponding wild-type strains. Interestingly,
21 rub-inoculation of the first leaves combined with symptom assessment by machine learning-based
22 imaging allowed significant distinction between wild-type and mutant strains. By contrast, dip-
23 inoculation of first trifoliolate leaves combined with chlorophyll fluorescence imaging did not
24 differentiate the strains. Furthermore, the new method developed here led to the miniaturization of
25 pathogenicity tests and significant time savings.

26

27 **INTRODUCTION**

28 Monitoring the impact of pathogens on plants is essential for improving knowledge on plant-
29 pathogen interactions and developing effective management practices (Bock et al., 2010).
30 Assessing plant symptoms is a key step in detecting plant resistance or evaluating the virulence of
31 a pathogen. Pathogenicity tests must give homogeneous and reproducible results and use an
32 objective method of symptom assessment to be interpretable (Nutter et al., 2006). Visual symptom
33 assessment is a simple and easily accessible method used in many studies of plant-pathogens
34 interactions. However, it lacks objectivity, accuracy and precision (Bock et al., 2008; Poland and

35 Nelson, 2010). The recent development of different optical techniques has allowed to automate the
36 process of symptom assessment while ensuring a standardization of results (Mahlein, 2016). As
37 such, computational image analysis provides a more objective, accurate, reproducible and
38 quantitative measure of disease severity than visual assessment.

39 Common bacterial blight of bean (CBB) is a significant bacterial disease on common bean, with
40 yield losses of more than 40% under favorable conditions (Belete and Bastas, 2017; Rodríguez De
41 Luque and Creamer, 2014). Symptoms can occur on leaves, stems, pods and seeds (Zaumeier and
42 Thomas, 1957). Leaf symptoms initially appear as water-soaked spots, which enlarge and can
43 coalesce with adjacent lesions (Goodwin and Sopher, 1994). Foliar lesions are often surrounded by
44 a chlorotic halo and evolve in necrosis, possibly resulting in the death of the entire leaf and partial
45 defoliation of the plant. Water-soaked spots and necrosis can also be observed on pods and seeds.
46 These symptoms evolve in dark red-brown lesions that are generally circular and slightly sunken
47 (Vidaver, 1993).

48 To phenotype CBB symptoms, different organs (seeds, pods, first leaves, trifoliolate leaves, stems)
49 can be inoculated in different ways. In particular, leaves can be inoculated by dipping, spraying,
50 rubbing, multiple needles or infiltration (Aggour et al., 1989; Popovic et al., 2012). Traditionally,
51 symptoms of CBB were assessed by visual evaluation using different rating scales based on a visual
52 estimation of the percentage of infected leaf area (Aggour et al., 1989; Cafati and Saettler, 1980;
53 Opio et al., 1993; Pastor-Corrales et al., 1981; Zapata, 2006). In 2012, it was shown that assessment
54 of CBB symptoms by RGB image analysis was more reproducible and more objective than a rating
55 scale, and presented a high differentiation power between plant genotypes (Xie et al., 2012).
56 Another method was developed based on leaf inoculation by dipping combined with chlorophyll
57 fluorescence imaging (Rousseau et al., 2013). This assessment method was successfully used to

58 discriminate different degrees of resistance in common bean against *Xpp-Xcf* (Foucher et al., 2020;
59 Rousseau et al., 2013).

60 CBB is caused by *Xanthomonas phaseoli* pv. *phaseoli* (*Xpp*) and *Xanthomonas citri* pv. *fuscans*
61 (*Xcf*) (Chen et al., 2021; Constantin et al., 2016). Pathological convergence of these two pathovars
62 is probably due to extensive horizontal gene transfers, which led to genomic regions sharing 100%
63 nucleotide identity between *Xpp* and *Xcf* (Aritua et al., 2015; Chen et al., 2018). These homologies
64 allowed the development of specific molecular tools for detecting both *Xpp* and *Xcf* on seed lots
65 (Audy et al., 1994; de Paiva et al., 2020; Grimault et al., 2014). Different *Xpp-Xcf* strains may have
66 different levels of pathogenicity regardless of whether they belong to one or the other pathovars
67 (Mkandawire et al., 2004). In addition, common bean resistance to CBB is mediated by numerous
68 quantitative trait loci (Monteiro et al., 2020; Singh and Miklas, 2015; Yu et al., 2012). Differences
69 in aggressiveness combined with the presence of quantitative resistances lead to a wide range of
70 possible disease intensities (Duncan et al., 2011). Thus, the interaction between common bean and
71 *Xpp-Xcf* must be finely phenotyped, in order to detect these variations as accurately as possible.

72 The pathogenicity of *Xanthomonas* is partly mediated by a type III secretion system (T3SS) and
73 associated type III effectors (T3Es) (An et al., 2019; Büttner and Bonas, 2010). Among T3Es,
74 *Xanthomonas* bacteria possess transcription activator-like effectors (TALEs). TALEs are injected
75 inside the plant cell via the T3SS and migrate to the nucleus where they are able to induce targeted
76 genes of the plant, often leading to disease enhancement (Boch and Bonas, 2010). Nine different
77 TALE-encoding genes and alleles were discovered in *Xpp-Xcf* (Ruh et al., 2017). *Xcf* strain 6165R
78 possesses only one *tal* gene named *tal22B* while *Xpp* strain 6546R bears two *tal* genes named *tal19I*
79 and *tal18H*.

80 In this study, we generated *tal* mutant strains 6165R Δ *tal22B* and 6546R Δ *tal18H*. Then, the
81 pathogenicity of strains 6165R and 6546R were compared to each other and to their corresponding

82 *tal* mutants. For this, we used two different tests to phenotype CBB symptoms in controlled
83 conditions. The first method corresponded to the method developed by Rousseau et al. (2013),
84 consisting of dipping the first trifoliolate leaf in bacterial suspension followed by chlorophyll
85 fluorescence imaging (CFI). The second method corresponded to a new pathogenicity test
86 consisting of rub-inoculation of the first leaves and evaluation of symptoms by machine learning-
87 trained imaging (MLI). MLI was performed on RGB images using the ilastik software (Berg et al.,
88 2019), which was recently exploited in biomedical and environmental studies as well as plant
89 symptoms assessment (Ilett et al., 2020; Ojeda-Martinez et al., 2020; Pike et al., 2020; Rashid et
90 al., 2019).

91

92 **MATERIALS AND METHODS**

93 **Bacterial strains and growth conditions**

94 *Xanthomonas phaseoli* pv. *phaseoli* strain 6546R and *Xanthomonas citri* pv. *fuscans* strain 6165R
95 are rifamycin-resistant derivatives of strains CFBP6546 and CFBP6165 respectively. Strains were
96 grown at 28 °C for 48 h on trypticase soy agar (TSA) medium (17.0 g.L⁻¹ pancreatic digest of
97 casein; 3.0 g.L⁻¹ enzymatic digest of soy bean; 5.0 g.L⁻¹ NaCl; 2.5 g.L⁻¹ K₂HPO₄; 2.5 g.L⁻¹
98 glucose; 15 g.L⁻¹ agar), then at 28 °C for 24 h on TSA10 (a 1/10 dilution of TSA, except for agar
99 maintained at 15 g.L⁻¹) to obtain fresh bacterial cultures. Media were supplemented by rifamycin
100 (50 mg. L⁻¹) for selection.

101

102 **Mutagenesis of *tal* genes and validation of mutants**

103 Plasmid pK18mob::sacB (Schäfer et al., 1994) was used to generate marker-free deletion mutants
104 of *tal22B* in strain 6165R or *tal18H* in strain 6546R. First, specific primers (Table S1) were
105 designed according to whole genome data (Ruh et al., 2017), to amplify the flanking regions of

106 each *tal* gene by PCR using the PHusion® High Fidelity DNA polymerase (Finnzymes, Waltham,
107 MA, USA) following the manufacturer's instructions. For each *tal* gene, PCR products were
108 purified using the Wizard® SV Gel and PCR Clean-Up System (Promega), then cloned in tandem
109 into the suicide plasmid pK18mob::sacB using digestion by *Bsa*I enzyme and ligation by T4 DNA
110 ligase. Recombinant plasmids were electrotransferred into competent 6165R or 6546R strains.
111 Primary transformants were immediately grown in liquid MOKA medium (yeast extract 4 g.L⁻¹;
112 casamino acids 8 g.L⁻¹; KH₂PO₄ 2 g.L⁻¹; MgSO₄.7H₂O 0.3 g.L⁻¹) for two hours at 28°C without
113 selection, then plated on MOKA agar medium supplemented with kanamycin (50 µg.mL⁻¹). Kan^r
114 colonies were then plated on MOKA medium supplemented with kanamycin and sucrose at 10%
115 for selecting secondary recombinants. Resulting colonies were tested for deletion of *tal* genes by
116 DNA extraction followed by PCR and sequencing.

117 Two additional analyses were done to validate *tal* mutants. First, to confirm that TALE proteins
118 corresponding to deleted genes were not produced, Western Blot assays were performed. Briefly,
119 total proteins were extracted from 0.4 mL of overnight bacterial suspensions and migrated by
120 Sodium Dodecyl Sulfate – Polyacrylamid Gel Electrophoresis (SDS-PAGE), then immunoblotted
121 with a primary anti-TALE antibody. The anti-TALE antibody was raised in rabbit against an *E.*
122 *coli*-produced designer TALE protein composed of the N-terminal domain of a TALE protein plus
123 six repeats (I. Fuentes and L. Noël, unpublished). TALE backbone was derived from the *hax3* tal
124 gene sequence from *X. campestris* pv. *campestris* strain Xca5 as described (Streubel et al., 2012).
125 Second, to check the influence of deletion on the growth capacity of the mutants, bacterial growth
126 control was performed. For this, liquid cultures at initial concentration of 1 × 10⁶ CFU.mL⁻¹ were
127 prepared in TS10 broth, and the absorbance (λ = 600nm) was monitored every 8 or 30 min using a
128 Labsystem Bioscreen C system, over an incubation period of 35 h at 28°C under shaking at 200
129 rpm. Each strain was controlled with technical triplicates and biological duplicates.

130

131 Genome sequencing, assembly and annotation

132 Genomic DNA of strains 6546R Δ *tal18H* and 6165R Δ *tal22B* was extracted with the Wizard[®]
133 Genomic DNA Purification Kit (Promega, Madison, USA) according to the manufacturer's
134 recommendations. PacBio Single Molecule Real Time (SMRT) sequencing was performed at the
135 Icahn School of Medicine at Mount Sinai (New York, USA) using one SMRT cell per strain to
136 achieve ~100× coverage. De novo assembling was performed using the following procedure. Reads
137 were filtered using PreAssembler Filter v1 of the SMRT Portal version 2.3 (Pacific Biosciences of
138 California, Inc.), with Minimum Subread Length 500, Minimum Polymerase Read Quality 0.80
139 and Minimum Polymerase Read Length 100. Assembly was performed using Canu v1.5 (Koren et
140 al., 2017). Circularisation was done using Berokka v0.2.3 (<https://github.com/tseemann/berokka>).
141 Sequence start was fixed using the Fixstart command of Circlator v1.5.1 (Hunt et al., 2015).
142 Polishing was performed using the variantCaller tool
143 (<https://github.com/PacificBiosciences/GenomicConsensus>) with --algorithm best. Whole genome
144 sequences of wild-type strains 6165R and 6546R (Briand et al., submitted) were used for
145 comparative analyses. Annotation of whole genome assemblies was performed with Prokka
146 v1.14.6 (Seemann, 2014). Average nucleotide identity analyses between genomes of wild-type and
147 TALE-deleted strains were performed with pyANI (Pritchard et al., 2016).

148

149 Plant materials and growing conditions

150 Seeds of the susceptible common bean cultivar JaloEEP558 were obtained from the Center for
151 Tropical Agriculture (CIAT, Colombia), available under accession number G9603
152 (<http://genebank.ciat.cgiar.org/genebank/main.do>). Plants were sown in plastic pots (7 × 7 × 8 cm)
153 containing pre-wetted soil. The sowings were covered with a P17 veil for four days for

154 homogenized germination. Plants were grown in a growth chamber at 23°C/20°C (day/night) with
155 a relative humidity of 80% and a photoperiod of 16 hours. Plants were watered every two days with
156 water for the first seven days, then with a nutrient solution (7.5-5-15 N-P-K) for up to 15 days, and
157 with a richer nutrient solution (15-10-30 N-P-K) until the end of the trial. The day before
158 inoculation, relative humidity and temperature were increased at 95% and 28°C/25°C (day/night)
159 to provide adequate conditions for infection. On the third day after inoculation, humidity was
160 reduced to 80% until the end of the assay.

161

162 **Pathogenicity assays**

163 For both methods, bacterial suspensions were calibrated at 1×10^7 CFU.mL⁻¹ in sterile distilled
164 water, and symptoms were monitored two weeks after inoculation. Each pathogenicity test was
165 performed twice independently.

166 For phenotyping by chlorophyll fluorescence imaging (CFI), inoculations were performed at stage
167 V1 (first trifoliolate leaf unfolded) by dipping the first trifoliolate leaf for 30 s into bacterial
168 suspensions or water as control. Symptom development was monitored by CFI at the PHENOTIC
169 Seeds and Plants platform of the IRHS in Angers (France) as described in Rousseau et al. (2013).
170 Briefly, inoculated leaflets were collected and set in the dark for 30 min. Then, for each leaflet, a
171 first picture was taken under a modulated flash of light to measure basal fluorescence (F₀) of the
172 tissues, followed by another picture taken under a high flash of saturating light to measure the
173 maximum fluorescence emission level (F_m). For each pixel, the maximum quantum yield of
174 photosystem II photochemistry ($F_v/F_m = (F_m - F_0)/F_m$) was calculated using Phenoplant
175 (<http://www.phenoplant.org>) to discriminate diseased and healthy leaflet areas (Rousseau et al.,
176 2015). For each plant, results correspond to the mean disease area percentage of the three leaflets
177 coming from the same inoculated leaf.

178 For phenotyping by machine learning-trained imaging (MLI), inoculations were performed by rub-
179 inoculation on the two first leaves of eight-day-old plants. Rub-inoculation consisted of one
180 passage of a gloved finger dipped in bacterial suspension or water as control. For each leaf, two
181 inoculations were done on the limb on each side of the central vein. To evaluate the symptoms,
182 detached leaves were put on a LED light table of 1,600 lumen and images were taken with a fixed-
183 height digital camera (Canon EOS 700D, Canon Inc., Taiwan) and saved as JPEG files. Each image
184 comprised the two first leaves of a same plant, each rubbed twice, thus representing four technical
185 replicates. Machine learning-based pixel segmentation was performed using the Pixel
186 Classification workflow from ilastik v1.3.3 (Berg et al., 2019). Training was performed using 14
187 features including color/intensity, edge and texture, on 37 images representative of the whole
188 dataset in terms of leaf colour and intensity of symptoms (training file available upon request).
189 Three labels were used for discriminating the background, leaf tissues and symptoms. Symptoms
190 corresponded to both chlorosis and necrotic tissues, as assessed by expert eye analysis. After the
191 training, batch processing of all images was performed. Pixel quantification was done using FiJi
192 (Schindelin et al., 2012). Briefly, labels were retrieved using the “Image threshold” option. Then,
193 the “Analyse particle” command was used to quantify pixels corresponding to either the
194 background or the symptoms. Total leaf areas were retrieved by subtracting background pixels
195 from the total image pixels.

196

197 **RESULTS**

198 **Phenotyping of strains with different degrees of pathogenicity**

199 For both inoculation methods, more severe symptoms appeared after inoculation of strain 6546R
200 than strain 6165R (Fig. 1). However, statistical distinction between strains was superior using rub-
201 inoculation with MLI ($p < 0.01$) than dip-inoculation with CFI ($p > 0.05$). The differences observed

202 between both methods could be explained by the inoculation method itself, which appeared as
203 playing a major role in the homogeneity of the symptoms. Indeed, dip inoculation of trifoliates led
204 to symptoms developing mainly from the margins, being unevenly distributed over the leaflets and
205 evolving into more or less extended patches hardly distinguishable between leaves inoculated by
206 one strain or the other (Fig. 1b and c). Moreover, the occurrence of symptoms was stochastic as
207 symptoms did not appear evenly on all leaflets of the same leaf, meaning that the variability
208 between leaflets was even higher than between individuals in some cases (not shown). In contrast,
209 symptoms were clearly distinguishable between both strains after rub inoculation (Fig. 1e and f).
210 For strain 6165R, symptoms appeared as tiny spots evenly distributed across the whole inoculated
211 area, likely caused by bacteria entering through openings corresponding to trichomes damaged by
212 the rubbing. For strain 6546R, most of the spots coalesced, leading to large symptomatic areas. In
213 all, rub-inoculation produced more homogeneous and reproducible symptoms than dip-inoculation.
214 To test if CFI could be used for assessing symptoms on rub-inoculated leaves, we compared CFI
215 and MLI after rub-inoculation of first leaves with strain 6165R (Supp. Fig. 1). While chlorotic areas
216 were accurately retrieved with both image acquisition methods, CFI failed to detect the tiny spots
217 corresponding to early symptoms. Moreover, CFI tended to take into account pixels outside the
218 inoculated area, thus not corresponding to symptoms caused by the bacteria (e.g. the petiole), which
219 was not the case for MLI. Therefore, MLI was more suited than CFI to detect symptoms on rub-
220 inoculated plants.

221

222 **Description of *tal* mutants**

223 To further evaluate the discriminating power of each symptom assessment method, we constructed
224 two deletion mutants of *tal* genes named 6546R Δ *tal18H* and 6165R Δ *tal22B*. Both 6546R Δ *tal18H*
225 and 6165R Δ *tal22B* were unable to produce TAL18H or TAL22B proteins, respectively (Fig. 2a

226 and b). PacBio SMRT sequencing allowed us to compare the complete genome sequences of the
227 mutants to their corresponding wild-type strains. In both cases, an average nucleotide identity of
228 more than 99.99% was found between the wild-type strains and their mutants, indicating that no
229 major modification occurred in the mutant strains.

230 As described before, both *tal22B* and *tal18H* are located on plasmids (Ruh et al., 2017). In strain
231 6165R Δ *tal22B*, the deletion of *tal22B* was restricted to a clean gap of 3,915 bp in plasmid A
232 corresponding to *tal22B* from start to stop (Fig. 2d). In strain 6546R, plasmid C comprised a gap
233 encompassing *tal18H* plus 2137 bp (Fig. 2c) including three genes encoding short (60 to 107
234 aminoacid-long) hypothetical proteins (not shown). For this strain, directed mutagenesis failed
235 until we tested more than 350 clones for *tal18H* deletion. The deletion was flanked by identical
236 *ISXac2* insertion sequences (IS) suggesting that it occurred through recombination between IS (Fig.
237 2c). Therefore, 6546R Δ *tal18H* corresponded to a variant of strain 6546R presenting a spontaneous
238 deletion of *tal18H* on plasmid C. For both strains, the deletion had no major effect on the bacterial
239 growth of the mutants compared to the wild-type strains in TS10 medium (Fig. 2e and f).

240

241 **Virulence evaluation of wild-type and mutant strains**

242 With both methods, the mutant strains led to less symptoms than the corresponding wild-type
243 strains, suggesting that *tal18H* and *tal22B* were involved in the pathogenicity of strains 6546R and
244 6165R, respectively (Fig. 3 and 4). However, rub-inoculation with MLI significantly discriminated
245 the two mutants from corresponding wild-type strains ($p < 0.05$), while dip-inoculation with CFI
246 did not ($p > 0.1$). Interestingly, the accuracy of rub-inoculation followed by MLI was high enough
247 to discriminate between strains presenting very low aggressiveness. Indeed, only 2% or 1% of the
248 total leaf pixels corresponded to symptoms after inoculation with 6165R or 6165R Δ *tal22B*,
249 respectively, which further demonstrated the discriminative power of this method.

250

251 Phenotyping of symptoms after leaf dipping

252 Although training was done on images of rub-inoculated primary leaves, we tested if MLI could
253 discriminate symptoms on trifoliolate leaves after inoculation by dipping. For this, we performed
254 both CFI and MLI on images taken from the same dip-inoculated plants (one image per leaflet).
255 We compared the CFI and MLI results to expert symptom assessment by eye using the reference
256 scale of Opio *et al.* (1993). Globally, MLI was able to detect most of the CBB symptoms retrieved
257 by eye, while CFI detected comparatively less visible symptoms than the two other methods (Supp.
258 Fig. 2). In accordance with this, correlation of visual assessment with MLI was stronger than with
259 CFI (Fig. 5). The high linear correlation found between visual assessment and MLI ($R^2 > 0.96$)
260 demonstrates that the MLI training developed in this study is a good estimator for symptom
261 quantification on dip-inoculated leaves.

262

263 DISCUSSION

264 Our study highlights that rub-inoculation on first leaves combined with MLI represents a fast,
265 simple and efficient way to quantify the fine symptom differences existing between strains
266 differing by only one or few genes. The two *tal* mutants constructed here induced less symptoms
267 than the wild-type strains, suggesting that TAL18H and TAL22B participate in the pathogenicity
268 of strains 6546R and 6165R, respectively. However, cloning of *tal* genes and complementation of
269 these strains is needed to confirm the role of these TALEs, especially because genes other than *tal*
270 were missing in strain 6546R Δ *tal18H*.

271 In addition to the gain of discriminating power, the rub-inoculation method presented several other
272 advantageous characteristics compared to dip-inoculation. First, the time required to carry out a
273 trial was reduced (Table 1). Indeed, the use of first leaves instead of first trifoliates saved one week

274 of plant growing time, leading to a 22-day trial instead of a month. Moreover, the growth of first
275 leaves was often more homogeneous than that of first trifoliolate leaves. Consequently, for a same
276 number of plants sown, more can be used for a pathogenicity test on first leaves than first trifoliate.
277 This can be an important factor for trials under controlled greenhouse conditions, where the space
278 available is often limited. In addition, the average inoculation time per plant (including all time
279 spent in the growth chamber) was three times longer for dip-inoculation than rub-inoculation
280 (Table 1), as it required more handling time and a 30-second immersion of the leaf in the bacterial
281 suspension. Furthermore, the volume of inoculum required was 20 times less for rub-inoculation
282 than dip-inoculation. Therefore, less material was needed for rub-inoculation and the waste was
283 easier to process. Finally, MLI requires simple materials such as a digital camera, a tripod and a
284 LED table, which can be afforded quite easily. On the other hand, CFI requires a complete
285 fluorescence imaging system that is much more expensive to purchase and maintain. The shooting
286 of plants (including handling time) was also more than three time longer for chlorophyll
287 fluorescence than RGB picture taking. This was mainly due to CFI requiring the leaves to be kept
288 in the dark for 30 minutes prior shooting, then perform the shootings leaflet by leaflet in a dark
289 room, while RGB pictures were done with both first leaves together.

290 Although the method developed here presents many advantages for symptom quantification, it is
291 important to note that chlorophyll fluorescence imaging is able to detect non-visible symptoms
292 resulting from a disruption of photosynthesis during the early stages of infection and has a potential
293 to discriminate between strains differing by a single gene (Méline et al., 2020). The future
294 development of pipelines combining different phenotyping methods will undoubtedly contribute
295 to analyze more in-depth the complex contributions of single or multiple genes to the virulence of
296 plant pathogens.

297

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314 ANAN platform from the SFR 4207 QUASAV (Angers, France). Bacterial strain preservation and
315 supply was ensured by the CIRM-CFBP (Beaucouzé, INRAE, France).

316

317 **DATA AVAILABILITY STATEMENT**

318 The data and materials that support the findings of this study (ilastik training file and bacterial
319 strains) are available from the corresponding author upon request. The whole genome sequences
320 of strains 6546R Δ *tal18H* and 6165R Δ *tal22B* were deposited to GenBank under accession numbers
321 CP072393-CP072395 and CP072396-CP072397, respectively.

322

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476

477 SUPPORTING INFORMATION LEGENDS

478 **Table S1.** List of primers used for *tal* mutagenesis

479 **Supplemental Figure 1:** Comparison between MLI and CFI after rub-inoculation of first leaves.

480 First leaves of bean plants were inoculated by rubbing with strain CFBP6165R. Images were taken
 481 14 days after inoculation. Each line shows the two first leaves of a same plant. Pixels corresponding
 482 to estimated symptoms are in red. MLI: machine learning-based imagery; CFI: chlorophyll
 483 fluorescence imagery.

484 **Supplemental Figure 2:** Comparison between MLI and CFI after dip-inoculation of trifoliolate

485 leaves. First trifoliolate leaves were inoculated with strains CFBP6165R, 6165RD*tal22B*,
 486 CFBP6546R, 6546RD*tal18H* or distilled water as control. Images were taken 12 days after

487 inoculation. For each condition, 15 leaflets coming from five different plants were analysed. **(a)**

488 Symptomatic areas measured using chlorophyll fluorescence imagery (CFI) or machine learning-

489 based imagery (MLI). **(b)** Images corresponding to the leaflets with symptoms closest to the mean

490 disease area as estimated by MLI in **(a)**. Pixels corresponding to estimated symptoms are in red.

491

492 **FIGURE LEGENDS**

493 **Figure 1.** Strain 6546R is more aggressive than strain 6165R. Images were taken at 14 days post
 494 inoculation, and evaluation of symptoms was done by chlorophyll fluorescence imaging on
 495 trifoliolate leaves inoculated by dipping **(a, b, c)**, or by machine learning-based imaging on first
 496 leaves inoculated by rubbing **(d, e, f)**. Experiments were repeated twice independently, with five
 497 plants per assay, and concatenated (n=10 plants). Box-plots represent the percentage of
 498 symptomatic area per plant and *p*-values were calculated by Mann-Whitney test. The images
 499 correspond to leaves or leaflets presenting the closest symptom percentages to the median values.
 500 The symptoms detected appear in red on the surface of the leaf in white. White bars correspond to
 501 1 centimeter.

502 **Figure 2.** Description of *tal* mutant strains 6546R Δ *tal18H* **(a, c, e)** and 6165R Δ *tal22B* **(b, d, f)**.
 503 Western blot analysis of TALEs **(a, b)**. Graphical map of plasmids from *tal* mutant strains using
 504 GView (Petkau et al., 2010) with corresponding wild-type strains as references **(c, d)**. The deletion
 505 observed in plasmid C from 6546R Δ *tal18H* corresponds to *tal18H* while the deletion in plasmid A
 506 from 6165R Δ *tal22B* corresponds to *tal22B* **(d)**. Bacterial growth dynamics of wild-type and mutant
 507 strains **(e, f)**. Growth assays were done as biological duplicates with technical triplicates. Bars
 508 represent standard deviation.

509 **Figure 3.** Pathogenicity of 6546R Δ *tal18H* compared to wild-type strain 6546R. Images were taken
 510 at 14 days post inoculation. Evaluation of symptoms was done by chlorophyll fluorescence imaging
 511 on trifoliolate leaves inoculated by dipping **(a, b, c)**, or by machine learning-based imaging on first
 512 leaves inoculated by rubbing **(d, e, f)**. Experiments were repeated twice independently, with eight
 513 plants per assay, and concatenated (n=16 plants). Box-plots represent the percentage of

514 symptomatic area per plant and p -values were calculated by Mann-Whitney test. The images
515 correspond to leaves or leaflets presenting the closest symptom percentages to the median values.
516 The symptoms detected appear in red on the surface of the leaf in white. White bars correspond to
517 1 centimeter.

518 **Figure 4.** Pathogenicity of 6165R Δ *tal22B* compared to wild-type strain 6165R. Images were taken
519 at 14 days post inoculation. Evaluation of symptoms was done by chlorophyll fluorescence imaging
520 on trifoliolate leaves inoculated by dipping (**a, b, c**), or by machine learning-based imaging on first
521 leaves inoculated by rubbing (**d, e, f**). Experiments were repeated twice independently, with five
522 plants per assay, and concatenated (n=10 plants). Box-plots represent the percentage of
523 symptomatic area per plant and p -values were calculated by Mann-Whitney test. The images
524 correspond to leaves or leaflets presenting the closest symptom percentages to the median values.
525 The symptoms detected appear in red on the surface of the leaf in white. White bars correspond to
526 1 centimeter.

527 **Figure 5.** Correlation between different symptom quantification methods. Trifoliolate leaves were
528 inoculated by dipping with either H₂O or strains 6165R, 6165R Δ *tal22B*, 6546R and
529 6546R Δ *tal18H*. For each leaflet, both chlorophyll fluorescence and RGB images were taken at 14
530 days post inoculation. Expert visual assessment of symptom percentage was performed on RGB
531 images using the scale by Opio et al. (1993) and results were compared to chlorophyll fluorescence
532 imaging (CFI, **a**) or machine learning-based imaging (MLI, **b**). The regression line formula and
533 correlation coefficient (R^2) were calculated with Excel, using 15 leaflets per condition (75 in total).

Table 1. Characteristics of pathogenicity tests.

	Trifoliolate dipping + CFI^a	First leaf rubbing + MLI^b
Discriminating power	Poor	Good
Detection of non-visible symptoms	Yes	No
Plant growing time	15 days	8 days
Symptom assessment date	14 days post inoculation	14 days post inoculation
Duration of a trial	29 days	22 days
Plants with homogeneous leaves	84%	93%
Average time of inoculation per plant	150 seconds	43 seconds
Volume per inoculum	500 mL	25 mL
Equipment	PSI Open FluorCam FC 800-O or equivalent	Light table and camera
Average image taking time per plant	327 seconds	45 seconds
Image analysis software	Phenoplant	Ilastik

^aCFI: chlorophyll fluorescence imaging

^bMLI: machine learning-based imaging

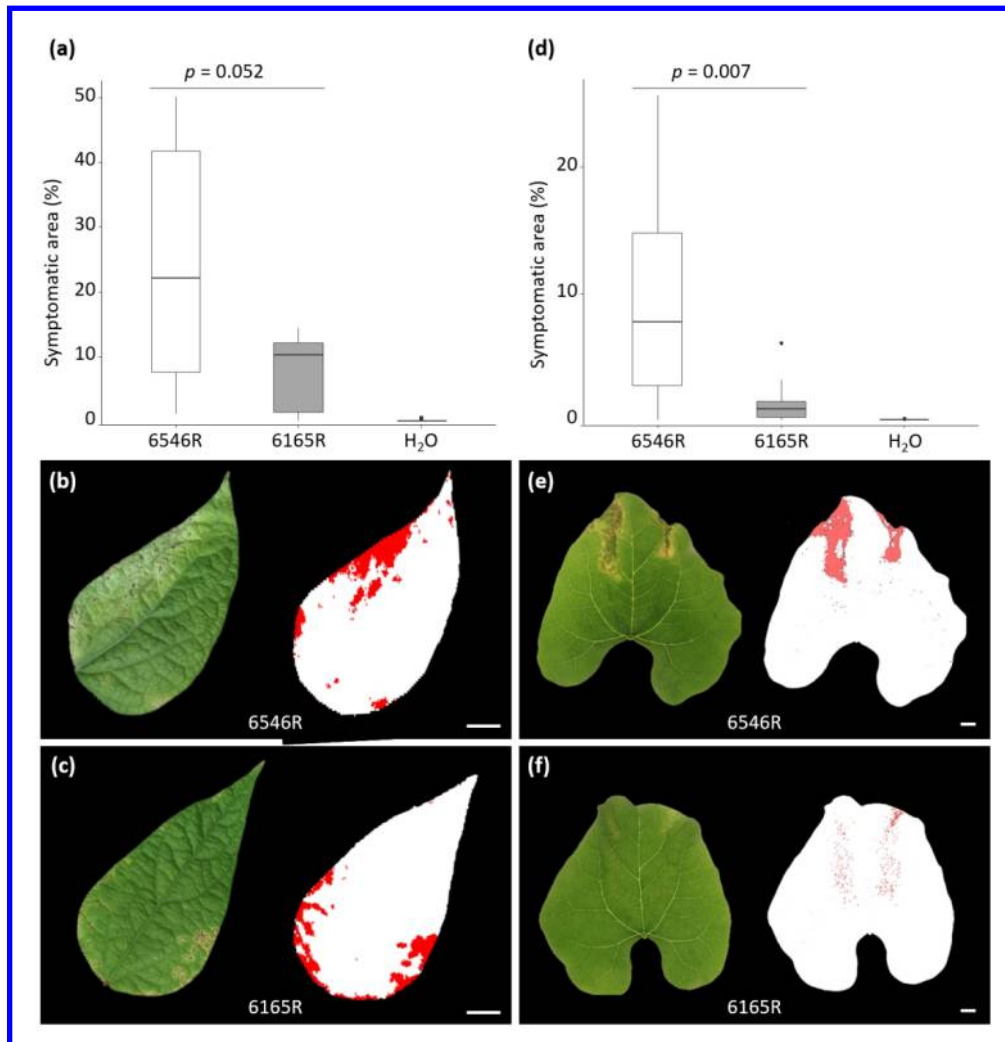


Figure 1. Strain 6546R is more aggressive than strain 6165R. Images were taken at 14 days post inoculation, and Evaluation of symptoms was done by chlorophyll fluorescence imaging on trifoliolate leaves inoculated by dipping (a, b, c), or by machine learning-based imaging on first leaves inoculated by rubbing (d, e, f). Experiments were repeated twice independently, with five plants per assay, and concatenated (n=10 plants). Box-plots represent the percentage of symptomatic area per plant and p-values were calculated by Mann-Whitney test. The images correspond to leaves or leaflets presenting the closest symptom percentages to the median values. The symptoms detected appear in red on the surface of the leaf in white. White bars correspond to 1 centimeter.

266x274mm (150 x 150 DPI)

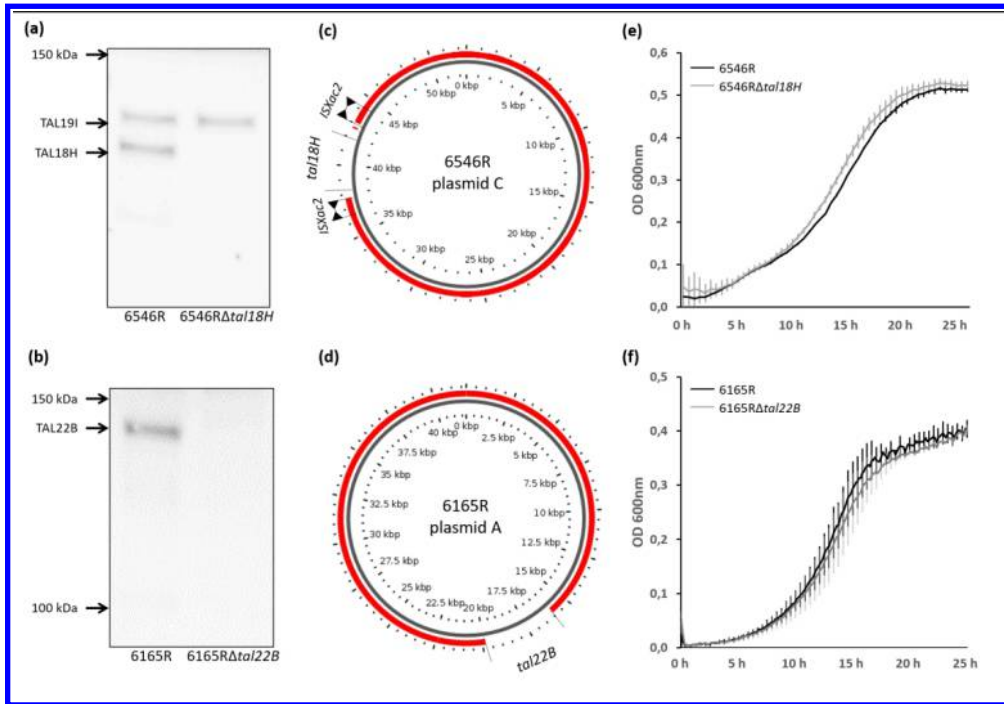


Figure 2. Description of tal mutant strains 6546RΔtal18H (a, c, e) and 6165RΔtal22B (b, d, f). Western blot analysis of TALEs (a, b). Graphical map of plasmids from tal mutant strains using GView (Petkau et al., 2010) with corresponding wild-type strains as references (c, d). The deletion observed in plasmid C from 6546RΔtal18H corresponds to tal18H while the deletion in plasmid A from 6165RΔtal22B corresponds to tal22B (d). Bacterial growth dynamics of wild-type and mutant strains (e, f). Growth assays were done as biological duplicates with technical triplicates. Bars represent standard deviation.

351x242mm (150 x 150 DPI)

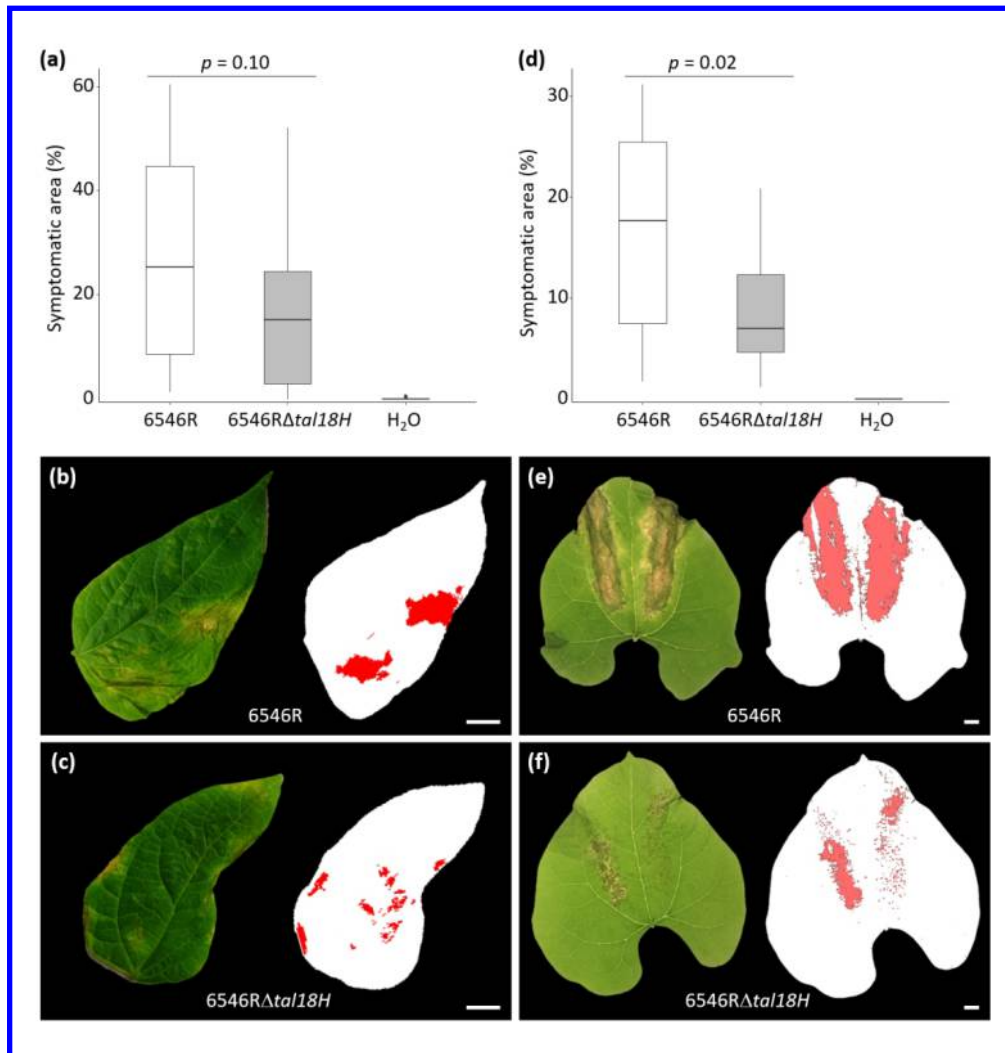


Figure 3. Pathogenicity of 6546R Δ tal18H compared to wild-type strain 6546R. Images were taken at 14 days post inoculation. Evaluation of symptoms was done by chlorophyll fluorescence imaging on trifoliolate leaves inoculated by dipping (a, b, c), or by machine learning-based imaging on first leaves inoculated by rubbing (d, e, f). Experiments were repeated twice independently, with eight plants per assay, and concatenated ($n=16$ plants). Box-plots represent the percentage of symptomatic area per plant and p-values were calculated by Mann-Whitney test. The images correspond to leaves or leaflets presenting the closest symptom percentages to the median values. The symptoms detected appear in red on the surface of the leaf in white. White bars correspond to 1 centimeter.

266x277mm (150 x 150 DPI)

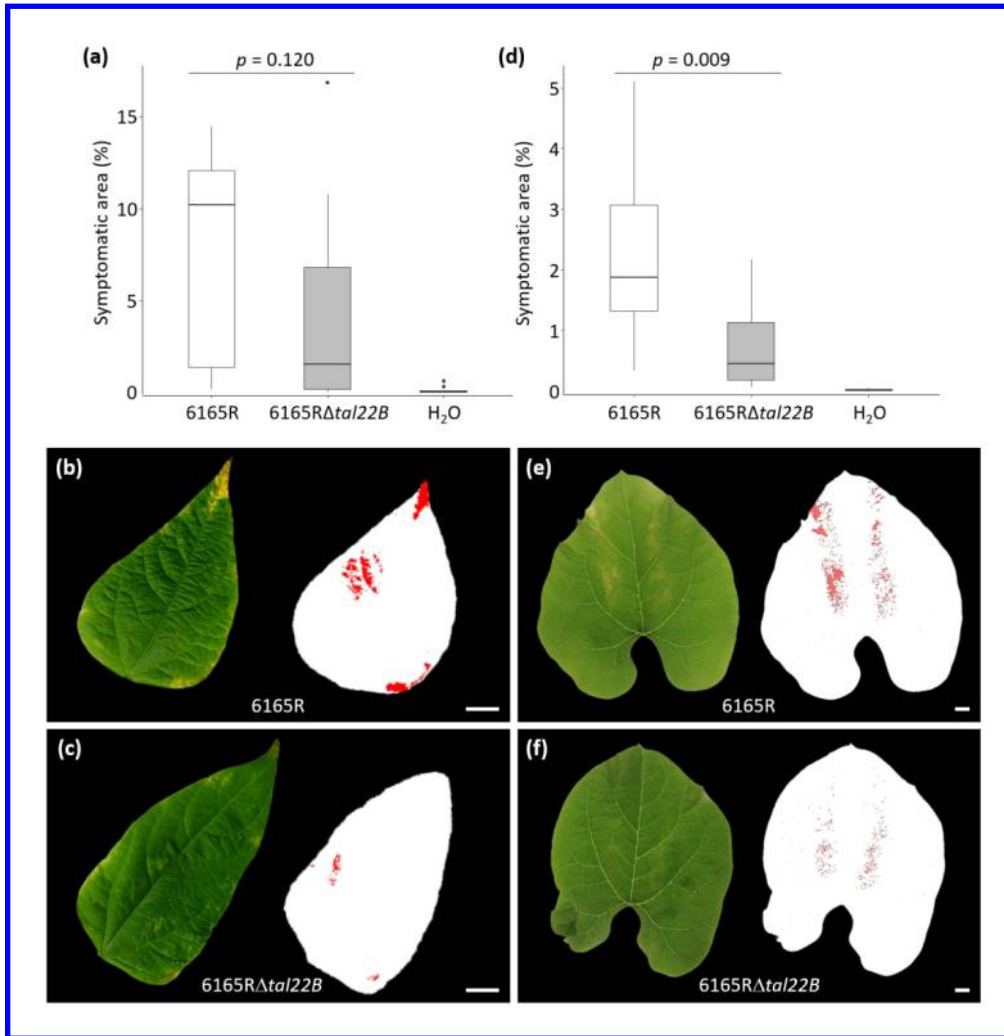


Figure 4. Pathogenicity of 6165RΔtal22B compared to wild-type strain 6165R. Images were taken at 14 days post inoculation. Evaluation of symptoms was done by chlorophyll fluorescence imaging on trifoliolate leaves inoculated by dipping (a, b, c), or by machine learning-based imaging on first leaves inoculated by rubbing (d, e, f). Experiments were repeated twice independently, with five plants per assay, and concatenated (n=10 plants). Box-plots represent the percentage of symptomatic area per plant and p-values were calculated by Mann-Whitney test. The images correspond to leaves or leaflets presenting the closest symptom percentages to the median values. The symptoms detected appear in red on the surface of the leaf in white. White bars correspond to 1 centimeter.

271x277mm (150 x 150 DPI)

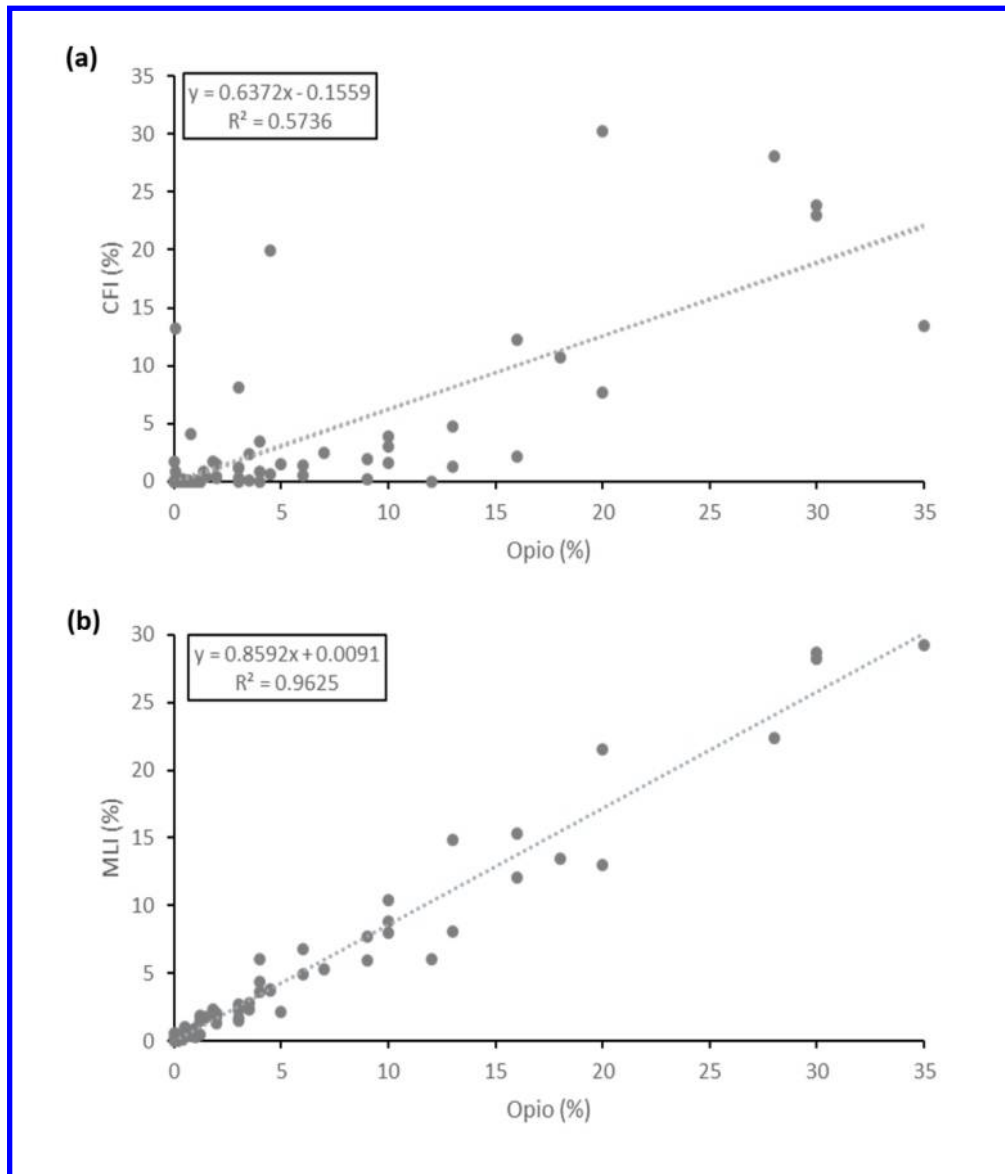
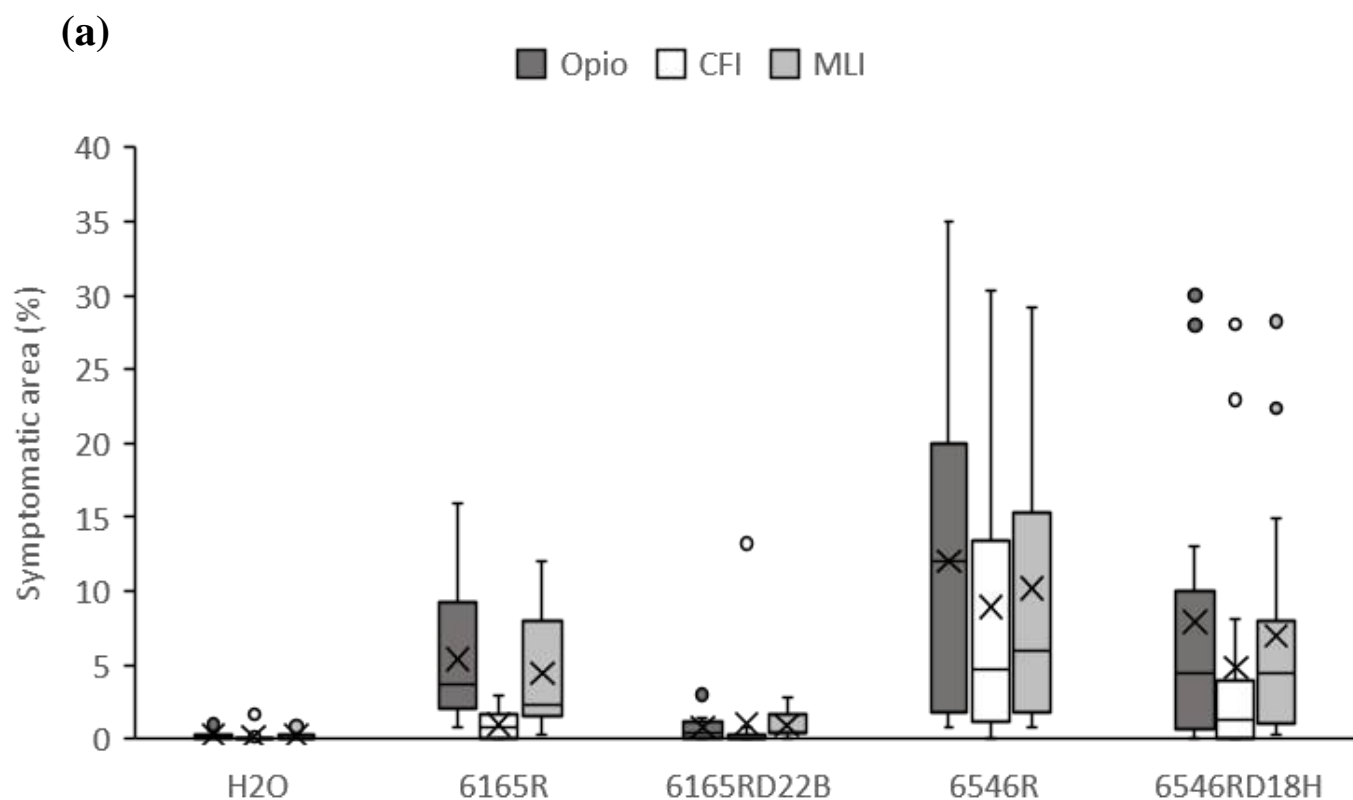


Figure 5. Correlation between different symptom quantification methods. Trifoliolate leaves were inoculated by dipping with either H₂O or strains 6165R, 6165R Δ tal22B, 6546R and 6546R Δ tal18H. For each leaflet, both chlorophyll fluorescence and RGB images were taken at 14 days post inoculation. Expert visual assessment of symptom percentage was performed on RGB images using the scale by Opio et al. (1993) and results were compared to chlorophyll fluorescence imaging (CFI, a) or machine learning-based imaging (MLI, b). The regression line formula and correlation coefficient (R²) were calculated with Excel, using 15 leaflets per condition (75 in total).

240x278mm (150 x 150 DPI)

Supplemental Figure 2: Comparison between MLI and CFI after dip-inoculation of first trifoliolate leaves. First trifoliolate leaves were inoculated with strains CFBP6165R, 6165R Δ *tal22B*, CFBP6546R, 6546R Δ *tal18H* or distilled water as control. Images were taken 12 days after inoculation. For each condition, 15 leaflets coming from five different plants were analysed. **(a)** Symptomatic areas measured using chlorophyll fluorescence imagery (CFI) or machine learning-based imagery (MLI). **(b)** Images corresponding to the leaflets with symptoms closest to the mean disease area as estimated by MLI in **(a)**. Pixels corresponding to estimated symptoms are in red.



MLI

RGB

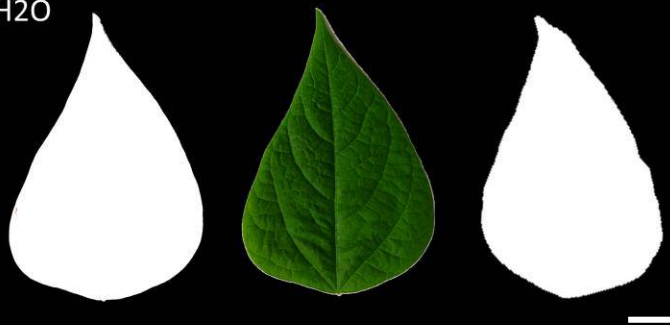
CFI

MLI

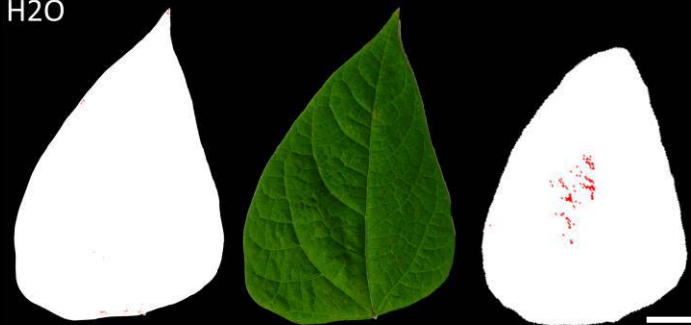
RGB

CFI

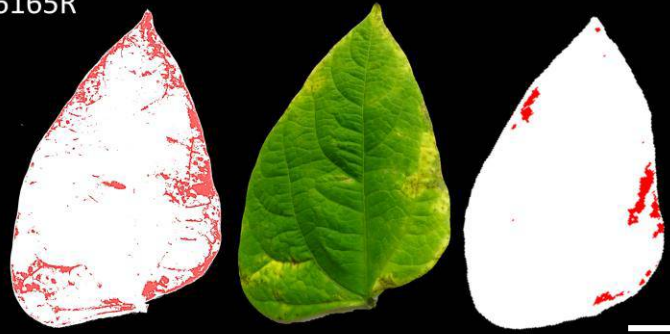
H2O



H2O



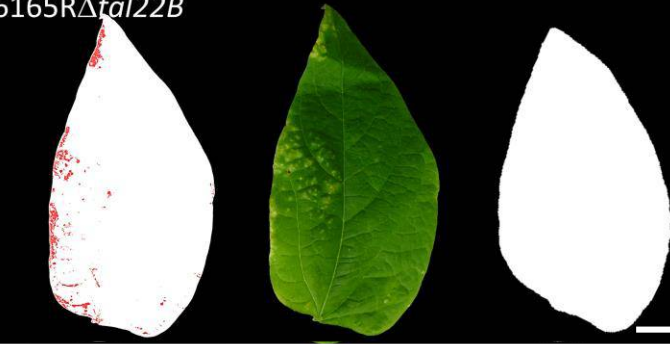
6165R



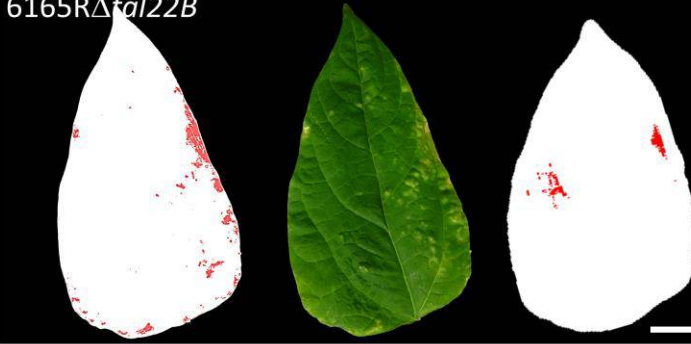
6165R



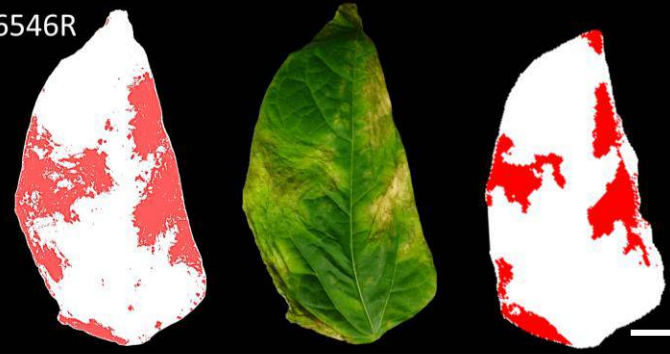
6165R Δ tal22B



6165R Δ tal22B



6546R



6546R



6546R Δ tal18H



6546R Δ tal18H

