Part 1: The identification of *Pseudomonas syringae* pathovars based on PR1a-GUS tobacco response

Introduction

Plants defend themselves against many disease-causing pathogens through innate and acquired resistance (Király et al., 2017). The innate plant resistance system relies on Pattern Recognition Receptors (PRRs) on the surface of the plant cell membranes. These perceive Pathogen-Associated Molecular Patterns (PAMPs) and induce PAMP-Triggered Immunity (PTI) in the host (Figure 1) (Zipfel, 2014; Bigeard et al., 2015). While PTI can provide resistance to some non-adapted pathogens, more specialised pathogens can deliver effector proteins through Type III Secretion System (T3SS) (Dodds and Rathjen, 2010; Cunnac et al., 2009), via pilus formation (Romantschuk et al., 2001), leading to Effector-Triggered Susceptibility (ETS) in the host.

Following the 'zig-zag' model of plant immunity (Jones and Dangl, 2006), plants can recognise effector proteins by nucleotide-binding leucine-rich repeats (NB-LRRs), encoded by *R* genes, which lead to Effector-Triggered Immunity (ETI). ETI leads to a stronger response compared to PTI, often resulting in localised programmed cell death (PCD) at the infection site, termed as hypersensitive response (HR), to stop further proliferation of the pathogen (Mittler et al., 1997). While T3SS is encoded by hypersensitive and pathogenicity (*hrp),* and conserved *hrp (hrc)* genes (Lindgren, 1997), the effector proteins are encoded by avirulence (*avr*) and *hrp* outer protein (*hop*) genes (Alfano and Collmer, 1997; Vencato et al., 2006).

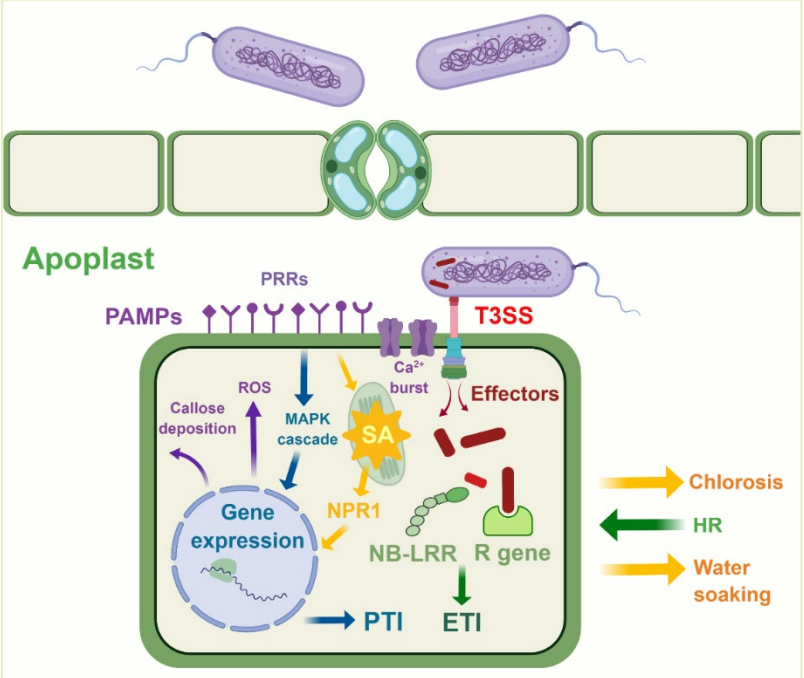

Figure 1. Interactions between phytopathogenic bacteria and the attacked host plant cells. Upon recognition of PAMPs with PRRs, PTI leads to ion fluxes across the plasma membrane, MAP kinase activation, production of ROS, changes in gene expression and callose deposition for plant cell wall reinforcement. The key regulator of the SA-dependent pathway is *NPR1* (Kumar, 2014). The bacteria secrete effectors via T3SS which are recognised by NB-LRRs, produced by R genes, leading to ETI in the host. Visible symptoms of successful pathogenesis are chlorosis and water soaking of the plant tissue. In response, HR by the host can limit further spread of the disease. Illustration by author. (PRRs - Pattern Recognition Receptors, PAMPs – Pathogen-Associated Molecular Patterns, PTI – PAMPs-Triggered Immunity, ROS – reactive oxygen species, SA – salicylic acid, NPR1 - *Nonexpresser of the PR protein 1,* T3SS – Type III Secretion System, NB-LRRs - nucleotide-binding leucine-rich repeats, ETI – Effector-Triggered Immunity, HR – Hypersensitive Reaction)

Studies on the genus *Pseudomonas* led to the discoveries of the pathways involved in the interactions between phyto-pathogenic bacteria and host plant tissues. It has been long debated how to treat the *P. syringae* species complex (Berge et al., 2014; Baltrus et al., 2017) but currently, 60 pathovars are accepted (Bull et al., 2010) which are divided into 13 phylogroups (Berge et al., 2014; Xin et al., 2018). T3SS in *P. syringae* (*Ps*) is encoded by *hrp/hrc* gene clusters, which have been termed as tripartite pathogenicity islands (Alfano et al., 2000).

Those *Ps* that are deficient in T3SS activate plant basal defences (i.e. PTI) while wild type *Ps* can suppress basal defences through effector proteins (i.e. ETS) (Nomura et al., 2005; Gimenez-Ibanez). It is also known that *Ps* colonises the xylem and can spread widely in in tobacco tissues (Misas-Villamil et al., 2011).

The virulence and growth within the plant is coordinated by the *hrpL* regulon, a member of the extracytoplasmic family of alternate sigma factors (Vencato et al., 2006), which also regulates *HrpA* gene that is responsible for pilus formation (Thwaites et al., 2004; Ortiz-Martin et al., 2010a). *HrpL* also regulates several genes which do not have T3SS-related functions such as the indoleacetate-lysine ligase gene, responsible for the biosynthesis of the coronatine (COR) phytotoxin (Lam et al., 2014).

COR consists of polyketide coronafacic acid and coronamic acid (Bender, 1999) and suppresses host defences (Nomura et al., 2005) and elicits stomatal opening (Melotto et al., 2006; Lee et al., 2013a; Arnaud and Hwang, 2015). COR mimics methyl jasmonate (MeJA) to activate the jasmonic acid (JA) signalling pathway (Nomura et al., 2005) which can counteract the salicylic acid (SA) mediated plant defence (Zheng et al., 2012; Lee et al., 2013b). This was shown by investigating the expression of SA-dependent *Pathogenesis-Related 1 (PR1)* genes in tobacco (Niki, 1998; Geng et al., 2014) and observing reduced disease severity in COR$^-$ mutants of *Ps* pathovars (Smirnova et al., 2002; Brooks et al., 2005). Certain *Ps* pathovars produce additional phyotoxins, such as tabtoxin by *Ps* pv. *tabaci (Pst)* (Glickmann et al., 1998) and phaseolotoxin by *Ps* pv. *phaseolicola (Psp)* (Xin et al., 2018).

While the host of *Pst* is tobacco (*Nicotiana tabacum*), the host of *Psp* is the common bean (*Phaseolus vulgaris*) (Figure 2). *Psp* induces *hrp*-dependent HR in tobacco (Mur et al., 2005). Based on interactions with different bean cultivars, nine *Psp* races have been described so far (Taylor et al., 1991; Arnold et al., 2011). The genomes of *Psp* and *Pst*, are 91.59-97.02% similar (Studholme et al., 2009) and belong to the *HopF1* subfamily (Lo et al., 2017). The effector *hopQ1-1,* is suggested to differentiate host specificity of *Psp* from *Pst* (Ferrante et al., 2009) while the disruption of the hairpin gene *hrpZ* may have led to the host specificity of *Pst* (Tsunemi et al., 2011).

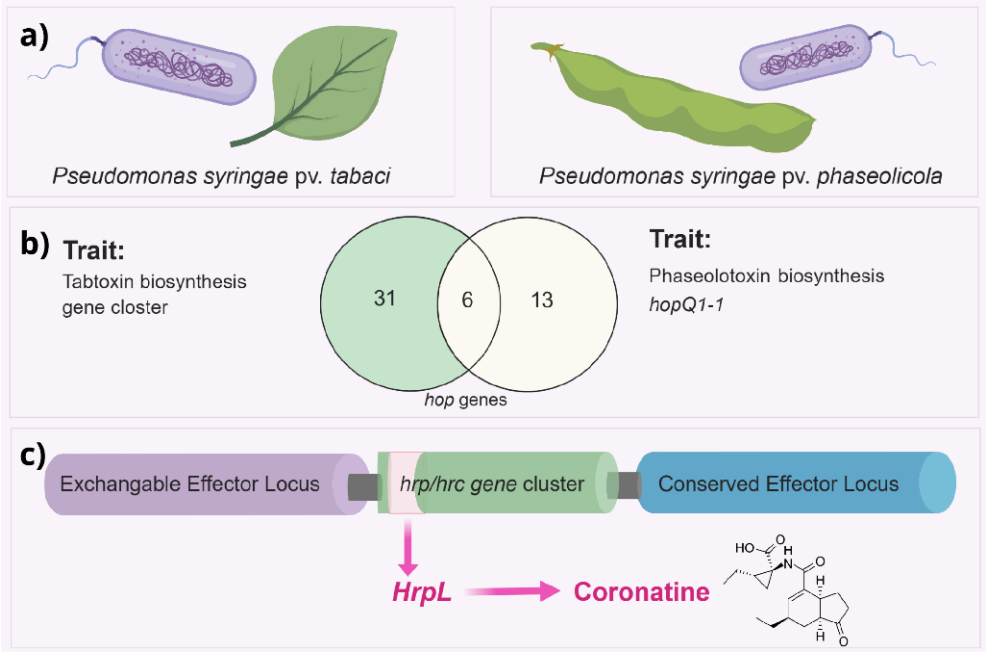

Figure 2. Comparison of *Pseudomonas syringae (Ps)* pv. *tabaci (Pst)* and pv. *phaseolicola (Psp)* phytotoxin biosynthesis gene cluster and the location of *HrpL* regulon. The pathovars infect different hosts (a), produce different phytotoxins while sharing *hop* genes (b) (Studholme et al., 2009). In *Ps*, the *hrp/hrc* gene clusters are flanked by conserved effector locus and exchangeable loci and *HrpL* regulon is regulates T3SS and coronatine production (c) (Lam et al., 2014).

In this study, we identified strains *Pst* and *Psp* and their mutants, *Pst* tabtoxin$^-$ and *Psp hrpL*$^-$ based visible host responses, bacterial colony counts and PR1a-GUS assay in *N. tabaci.* We expected *Psp hrpL*$^-$ non-host strain to be unable to translocate effectors, while the *Pst* tabtoxin$^-$ strain was expected to successfully translocate effectors but only induce chlorosis (Figure 3). We expected a stronger response in HR to *Psp* compared to *Pst* and therefore higher PR1a-GUS activity.

	PTI	ETS	ETI	vir$^+$	vir$^-$
Psp	+	+	+	+	-
Psp hrpL$^-$	+	-	-	+	-
Pst tab$^+$	+	+	-	+	-
Pst tab$^-$	+	+	-	-	+

Figure 3. Expectations of host response to the strains and their mutants used in this study. PTI - PAMP-Triggered Immunity, ETS - Effector-Triggered Susceptibility, ETI - Effector-Triggered Immunity, *Psp* - *Pseudomonas* pv. *phaseolicola, Pst* - *Pseudomonas* pv. *tabaci,* vir – virulence, tab – tabtoxin.

Methods

Rifampicin-resistant (rif) (Staskawicz et al., 1984) strains 'A', 'B', 'C', 'D' and control 'M' (10 mM $MgCl_2$) were infiltrated according to Mur et al. (1996) on five PR1a-GUS *Nicotiana tabaci* plants (Warner et al., 1993; Bi et al., 1995). Three leaf cores were taken from each infected plant with 1.5 ml Eppendorf Tubes® and bacterial counts were carried out after five days post infiltration (dpi) (Mur et al., 1996). GUS activity assay (Warner et al.,1992) was expressed as pmol 4-MU (4-methylumbelliferone) min^{-1}/core. All statistical analyses were carried out in R software (R Development Core Team; https://www.r-project.org/) using 'ggplot' package (Wickham, 2016).

Results

HR response was observed in PR1a-GUS tobacco plants infiltrated with strain 'A', where the necrotic lesion edge was serrated showing signs of disease spread from the inoculation site (Figure 4). No HR response was seen in tobacco plants inoculated with strain 'B', only signs of spreading chlorosis from the inoculation. HR response was observed in response to strains 'C' and 'D' and the necrotic lesion edges were smooth. HR response was most extreme in response to strain 'D' where the leaf surface became wrinkled. Based on Mur et al. (2005)'s *Ps* pv. *tabaci* lesion phenotype scoring, the host responses were not stronger than Score 2.

Bacterial colony counts per infected tobacco leaf core were on average 3.2×10^4 (95% CIs [2.3×10^4, 4×10^5]) for strain 'A'(Figure 5a), and 9.3×10^4 (95% CIs [7.9×10^4, 1.06×10^5]) for strain 'B'. For strain 'C' colony counts were on average 5.1×10^4 (95% CIs [4.09×10^4, 6.1×10^4]) and on average 1.6×10^4 (95% CIs [1.4×10^4, 1.8×10^4]) colonies were recovered for strain 'D'. One-way ANOVA found a statistically significant difference ($F = 15.46$, $df = 3$, $P = 0.001$) between the bacterial colony numbers and strains. Tukey's honestly significant difference (HSD) post hoc test found significant differences between strains 'A' and 'B' ($P = 0.004$), 'B' and 'C' ($P = 0.033$) and 'B' and 'D' ($P = 0.001$).

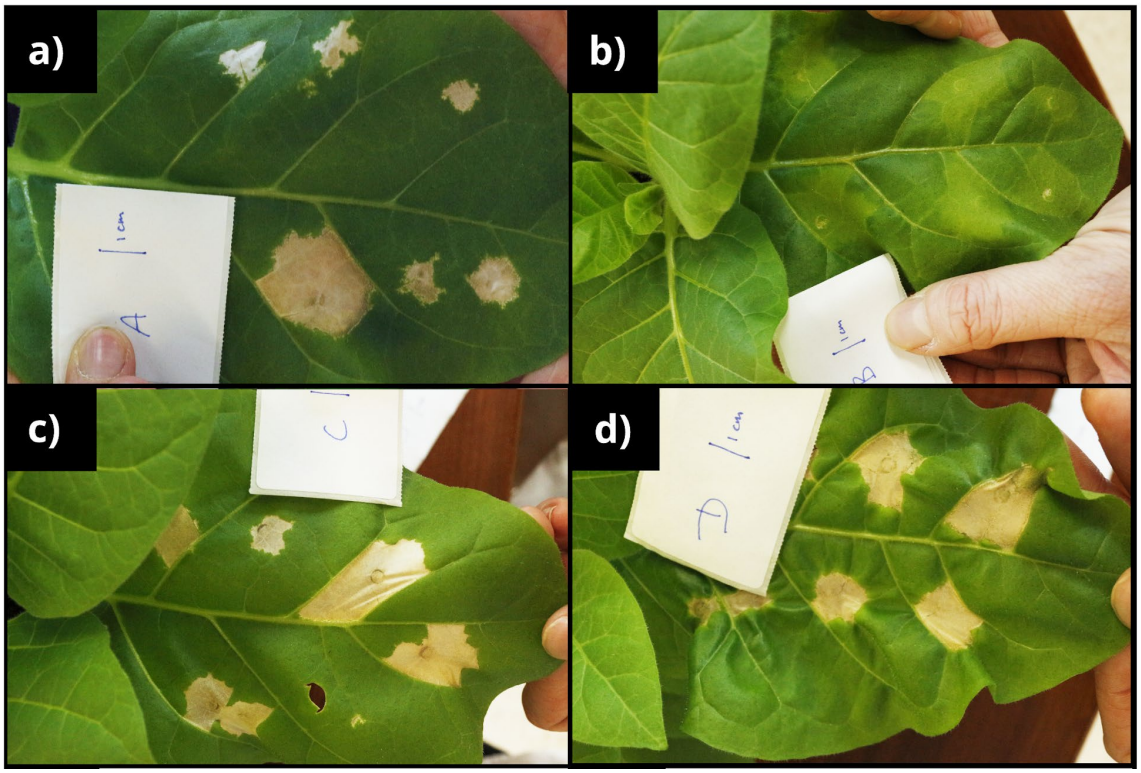

Figure 4. Infiltrations with strain 'A'(a), 'B' (b), 'C' (c) and 'D' (d) on tobacco leaves. While strain 'B' did not elicit hypersensitive response, strain 'D' appeared to lead to the strongest host response.

PR1-a GUS expression in tobacco inoculated with strain 'A' was on average 1,897.5 pmol 4 MU min^{-1}/core (95% CIs [758.11, 3036.89]) (Figure 5b), and on average 458.33 pmol 4 MU min^{-1}/core (95% CIs [0.00, 1231.02]) for strain 'B'. In tobacco inoculated with strain 'C', GUS activity was on average 2,190.83 pmol 4 MU min^{-1}/core (95% CIs [905.66, 3476.01]) and for strain 'D', GUS activity was on average 3,694.16 pmol 4 MU min^{-1}/core (95% CIs [3075.444, 4312.89]). One-way ANOVA found a statistically significant difference (F = 7.84, df = 4, P = 0.004) between PR1a-GUS activity and different strains. However, HSD post hoc test only found significant differences between strains 'B'-'D' (P = 0.01) and 'D'-'M' (P = 0.0039).

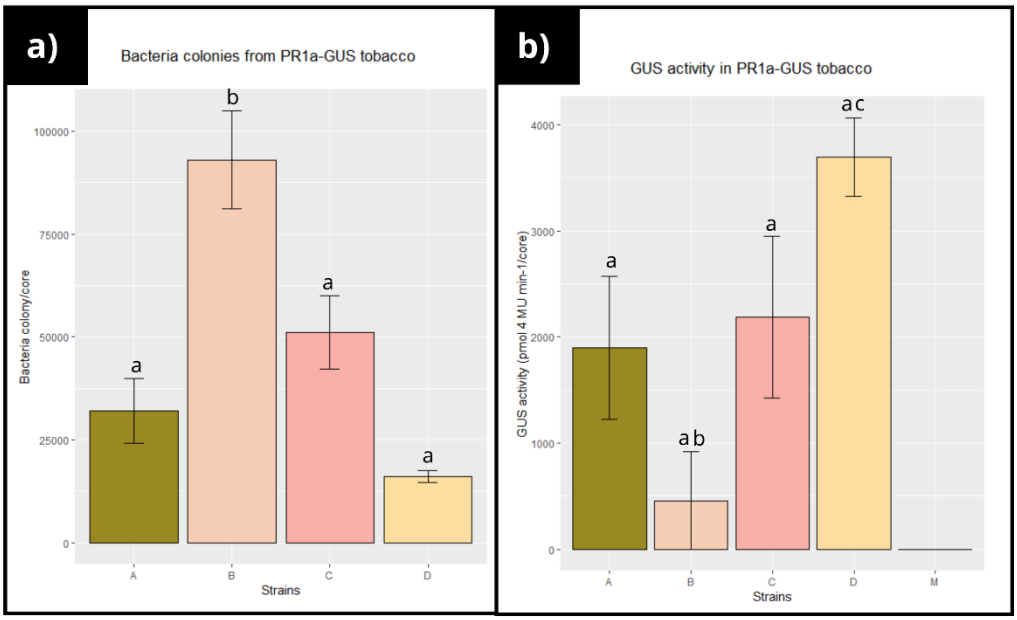

Figure 5. Bacterial colony counts from leaf cores (a) and PR1a-GUS activity (b) in tobaccos infiltrated with strains 'A', 'B', 'C' and 'D'. ANOVA tests showed that colony counts recovered from tobaccos infiltrated with strain 'B' were the only statistically significantly (P<0.05) different from the other strains.

Discussion

I suggest strain 'D' to be *Ps* pv. *phaseolicola (Psp)* as it caused the most extreme HR which has also elicited the highest PR1a-GUS activity. As I expected, the low PR1a-GUS activity in tobaccos infiltrated with strains 'A', 'B' and 'C' in comparison to strain 'D' reflected ETI as reported by Giemenez-Ibanez et al. (2018). I identify strain 'B' to be the *Psp hrpL*$^-$ mutant as it only induced chlorosis, reflecting its incapability of delivering effectors through T3SS. I identify strain 'A' as *Ps* pv. *tabaci (Pst)* tabtoxin$^-$ mutant due to the lack of chlorosis and serrated necrotic lesion edge. Although no significant difference was found in between strains 'A' and 'C' in terms of bacterial colony count and PR1a-GUS activity, the chlorosis around strain 'C' lesions indicates it to be *Pst*.

The host response to *Pst* and *Psp* was not as strong as described previously (Mur et al., 2000; Mur et al., 2005) but strain 'C' lesions *(Pst)* appeared to spread from the lesions which was previously observed for *Pst* on *N. benthamiana* using chlorophyll *a* fluorescence imaging (Iqbal et al., 2012). I did not observe any chlorosis around the strain 'A' lesions (*Pst* tabtoxin$^-$), only serrated necrotic lesion edges. Chlorosis is typically caused by the tabtoxin itself (Turner, 1988; Glickmann et al., 1998) leading to chlorophyll degradation (Cheng et al., 2016). Previous reports either did not detect visible symptoms or observed reduced virulence of *Pst* tabtoxin$^-$ mutants on tobacco (Turner and Taha, 1984; Marutani et al., 2008; Barta et al., 1992). While tabtoxin has been established to be complimentary to *Ps* virulence (Turner and Taha, 1984; Salch and Shaw, 1988; Barta et al., 1992; Bender et al., 1999; Arrebola et al., 2011), we did not detect more bacteria colony numbers or reduced SA accumulation when comparing *Pst* and *Pst* tabtoxin$^-$ mutants. Chlorosis has been attributed to ammonia production in tabtoxin-treated tissue which stops in dark conditions (Turner and Debbage, 1982), so the role of light could have been a confounding factor. Using spray-infiltration (Marutani et al., 2008) instead of a syringe for infiltration could also improve the experiment (Gottwald and Graham, 1992). As tabtoxin can be identified using polymerase chain reaction (PCR) amplifications of the respective genes (Lydon and Patterson, 2001), PCR could be used to differentiate *Pst* and *Pst* tabtoxin$^-$ strains.

In comparison to *Pst*, *Psp* is unable to spread within the non-host plant (Mur et al., 2000). Bacterial colony numbers were the lowest for *Psp* (strain 'D') while Pr1a-GUS activity was the highest, indicating that it was a non-host strain, and its HopQ1 (*Hrp* outer protein Q) effectors were recognised by the host leading to ETI (Mur et al., 2000; Giska et al., 2013). The *Psp hrpL*$^-$ mutant was similarly non-HR reducing as reported in previous studies (Bestwick et al., 1997; Kenton et al., 1999; Klement et al., 1999; Ortiz-Martin et al., 2010a; Kiba et al., 2018). As the bacterial colony counts were the highest for the *Psp hrpL*$^-$ mutant (strain 'B') and PR1a-GUS activity was the lowest, these bacteria appeared to successfully spread within a host but only inducing PTI (Kiba et al., 2018). Our bacterial colony counts were similar to those of Ortiz-Martin et al.'s (2010b) *Psp hrpL*$^-$ mutants 14 days dpi suggesting that suspensions used for infiltration were highly concentrated. The low PR1a-GUS activity was consistent with Huang et al.'s (2003) study where *Ps* pv. *tomato hrpC*$^-$ mutant did not trigger SA accumulation. While *Psp hrpD*$^-$ mutant on lettuce led to HR, the host response was very localised and the bacteria were unable to multiply (Bestwick et al., 1995).

While the present study relied on visual assessment, bacterial colony counts and PR1a-GUS activity observations, tabtoxin and phaseolotoxin could be identified using BOX-PCR and DNA/DNA hybridisation with nutritional characterisations (Abi et al., 2000). Overall, we were able to identify the least (*Psp hrpL*$^-$*)* and most extreme *(Psp)* host responses but found bacterial colony counts and PR1a-GUS activity were not reliable to distinguish *Pst* and *Pst* tabtoxin$^-$.

Part 2: Salicylic acid as a tobacco defence mechanism against Tobacco Mosaic Virus (TMV)

Salicylic acid (SA) is synthesised via the phenylalanine ammonia-lyase (PAL) mediated pathway and the isochromatic synthase catalysed pathway (Janda and Ruelland, 2015). Its production is associated with *Pathogenesis Related (PR)* gene induction which then produce vacuole-targeted proteins against pathogens (Dong, 2004). Tobacco Mosaic Virus (TMV) single stranded RNA virus with a longevity of 3,000 days within the plant sap (Creager et al., 1999). Tobacco plants overexpressing a bacterial *salicylate hydroxylase* (*NahG*) gene, convert SA to catechol and as a result, the SA levels are very low in the transformed plants (van Wees et al., 2003). Here, we investigated the host responses of PR1a-GUS and NahG tobaccos in response to TMV, using Mur et al.'s methods (1996).

Results

Growth rings were seen around the lesions on the PR1a-GUS tobaccos (Figure 6a) while there were more lesions on the 35S-NahG plants (Figure 6b). The average lesion size from three PR1a-GUS tobacco was 0.158 mm (95% CIs [0.146, 0.171]) and on the 35S-NahG tobacco plants, average lesion size was 0.139 mm (95% CIs [0.136, 0.143]). These were not statistically significant different (t = 0.95305, df = 10.298, P = 0.3624) (Figure 6c).

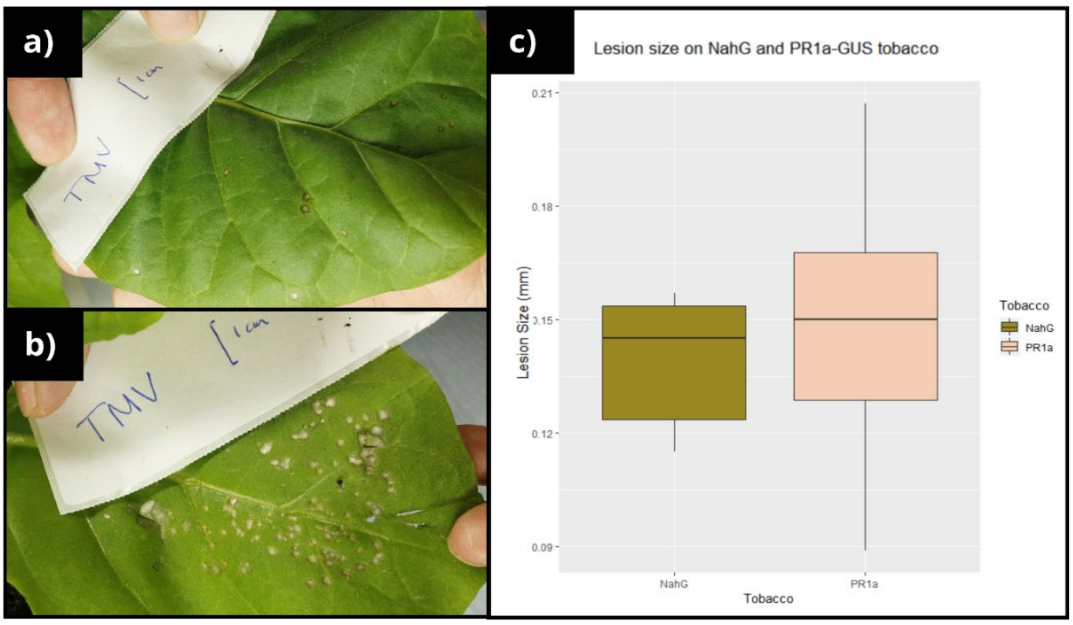

Figure 6. Tobacco plants PR1a-GUS (a) and NahG (b) inoculated with TMV. The lesion sizes did not significantly differ (c) but it was visible that NahG plants had more necrotic lesions while PR1-GUS plants had fewer lesions with visible growth rings. Three replicates were used in this study.

Prediction

While PR1 protein accumulation requires 3-7 days to defend through the SA-mediated pathway, our three (strains 'A', 'C', and 'D') previous *Psp* and *Pst* inoculations elicited necrotic lesions in less than five days. This suggests that if we were to inoculate 35S-NahG with the three strains, we would have observed rapid bacterial spread (e.g. within less than 2 hours) while PR1a-GUS activity would have been negligible as the host defence pathway would have been degraded (Jia et al., 2016). Infiltration with strain 'B' (*Psp hrpL*$^{-}$ mutant) would have led to even higher bacterial colony numbers but would have stayed non-HR inducing. However, the actual action of *PR1a* is not well defined despite being accepted as a SA-mediated plant defence gene (Rayapuram et al., 2008; Gupta et al., 2013). For TMV resistance breeding purposes *N* gene mediated provides more reliable results (Yu et al., 2017).

Word count: 2,234

References

Abi, S.D.A., Corbière, R., Gardan, L., Tourte, C., Manceau, C., Taylor, J.D. and Samson, R., 2000. Multiphasic approach for the identification of the different classification levels of *Pseudomonas savastanoi* pv. *phaseolicola*. *European Journal of Plant Pathology*, *106*(8), pp.715-734.

Alfano, J.R. and Collmer, A., 1997. The type III (Hrp) secretion pathway of plant pathogenic bacteria: trafficking harpins, *Avr* proteins, and death. *Journal of Bacteriology*, *179*(18), p.5655.

Arnaud, D. and Hwang, I., 2015. A sophisticated network of signaling pathways regulates stomatal defenses to bacterial pathogens. *Molecular Plant*, *8*(4), pp.566-581.

Arnold, D.L., Lovell, H.C., Jackson, R.W. and Mansfield, J.W., 2011. *Pseudomonas syringae* pv. *phaseolicola*: from 'has bean' to supermodel. *Molecular Plant Pathology*, *12*(7), pp.617-627.

Arrebola, E., Cazorla, F.M., Perez-García, A. and Vicente, A.D., 2011. Chemical and metabolic aspects of antimetabolite toxins produced by *Pseudomonas syringae* pathovars. *Toxins*, *3*(9), pp.1089-1110.

Baltrus, D.A., McCann, H.C. and Guttman, D.S., 2017. Evolution, genomics and epidemiology of *Pseudomonas syringae*: challenges in bacterial molecular plant pathology. *Molecular Plant Pathology*, *18*(1), pp.152-168.

Barta, T.M., Kinscherf, T.G. and Willis, D.K., 1992. Regulation of tabtoxin production by the *lemA* gene in *Pseudomonas syringae. Journal of Bacteriology*, *174*(9), pp.3021-3029.

Bender, C.L., Alarcón-Chaidez, F. and Gross, D.C., 1999. *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. *Microbiology Molecular Biology Reviews*, *63*(2), pp.266-292.

Berge, O., Monteil, C.L., Bartoli, C., Chandeysson, C., Guilbaud, C., Sands, D.C. and Morris, C.E., 2014. A user's guide to a data base of the diversity of *Pseudomonas syringae* and its application to classifying strains in this phylogenetic complex. *PloS One*, *9*(9), p.e105547.

Bestwick, C.S., Bennett, M.H. and Mansfield, J.W., 1995. Hrp mutant of *Pseudomonas syringae* pv *phaseolicola* induces cell wall alterations but not membrane damage leading to the hypersensitive reaction in lettuce. *Plant Physiology*, *108*(2), pp.503-516.

Bestwick, C.S., Brown, I.R., Bennett, M.H. and Mansfield, J.W., 1997. Localization of hydrogen peroxide accumulation during the hypersensitive reaction of lettuce cells to *Pseudomonas syringae* pv. *phaseolicola. The Plant Cell*, *9*(2), pp.209-221.

Bi, Y.M., Kenton, P., Mur, L., Darby, R. and Draper, J., 1995. Hydrogen peroxide does not function downstream of salicylic acid in the induction of PR protein expression. *The Plant Journal*, *8*(2), pp.235-245.

Bigeard, J., Colcombet, J. and Hirt, H., 2015. Signaling mechanisms in pattern-triggered immunity (PTI). *Molecular Plante*, *8*(4), pp.521-539.

Brooks, D.M., Bender, C.L. and Kunkel, B.N., 2005. The *Pseudomonas syringae* phytotoxin coronatine promotes virulence by overcoming salicylic acid-dependent defences in Arabidopsis thaliana. *Molecular Plant Pathology*, *6*(6), pp.629-639.

Bull, C.T., De Boer, S.H., Denny, T.P., Firrao, G., Saux, M.F.L., Saddler, G.S., Scortichini, M., Stead, D.E. and Takikawa, Y., 2010. Comprehensive list of names of plant pathogenic bacteria, 1980-2007. *Journal of Plant Pathology*, pp.551-592.

Cheng, D.D., Zhang, Z.S., Sun, X.B., Zhao, M., Sun, G.Y. and Chow, W.S., 2016. Photoinhibition and photoinhibition-like damage to the photosynthetic apparatus in tobacco leaves induced by *Pseudomonas syringae* pv. *tabaci* under light and dark conditions. *BMC Plant Biology*, *16*(1), p.29.

Creager, A.N., Scholthof, K.B.G., Citovsky, V. and Scholthof, H.B., 1999. Tobacco mosaic virus: pioneering research for a century. *The Plant Cell, 11,* pp.301-308.

Cunnac, S., Lindeberg, M. and Collmer, A., 2009. Pseudomonas syringae type III secretion system effectors: repertoires in search of functions. *Current Opinion in Microbiology*, *12*(1), pp.53-60.

Dodds, P.N. and Rathjen, J.P., 2010. Plant immunity: towards an integrated view of plant–pathogen interactions. *Nature Reviews Genetics*, *11*(8), p.539.

Dong, X., 2004. NPR1, all things considered. *Current Opinion in Plant Biology*, *7*(5), pp.547-552.

Ferrante, P., Clarke, C.R., Cavanaugh, K.A., Michelmore, R.W., Buonaurio, R. and Vinatzer, B.A., 2009. Contributions of the effector gene *hopQ1-1* to differences in host range between *Pseudomonas syringae* pv. *phaseolicola and P. syringae* pv. *tabaci. Molecular Plant Pathology*, *10*(6), pp.837-842.

Geng, X., Jin, L., Shimada, M., Kim, M.G. and Mackey, D., 2014. The phytotoxin coronatine is a multifunctional component of the virulence armament of *Pseudomonas syringae. Planta*, *240*(6), pp.1149-1165.

Gimenez-Ibanez, S., Hann, D.R., Chang, J.H., Segonzac, C., Boller, T. and Rathjen, J.P., 2018. Differential suppression of *Nicotiana benthamiana* innate immune responses by transiently expressed *Pseudomonas syringae* type III effectors*. Frontiers in Plant Science, 9*, article.688.

Giska, F., Lichocka, M., Piechocki, M., Dadlez, M., Schmelzer, E., Hennig, J. and Krzymowska, M., 2013. Phosphorylation of *HopQ1,* a type III effector from *Pseudomonas syringae*, creates a binding site for host 14-3-3 proteins. *Plant Physiology*, *161*(4), pp.2049-2061.

Gottwald, T.R. and Graham, J.H., 1992. A device for precise and nondisruptive stomatal inoculation of leaf tissue with bacterial pathogens. *Phytopathology*, *82*(9), pp.930-935.

Huang, J., Cardoza, Y.J., Schmelz, E.A., Raina, R., Engelberth, J. and Tumlinson, J.H., 2003. Differential volatile emissions and salicylic acid levels from tobacco plants in response to different strains of *Pseudomonas syringae*. *Planta*, *217*(5), pp.767-775.

Iqbal, M.J., Goodwin, P.H., Leonardos, E.D. and Grodzinski, B., 2012. Spatial and temporal changes in chlorophyll fluorescence images of *Nicotiana benthamiana* leaves following inoculation with *Pseudomonas syringae* pv. *tabaci. Plant P athology*, *61*(6), pp.1052-1062.

Janda, M. and Ruelland, E., 2015. Magical mystery tour: salicylic acid signalling. *Environmental and Experimental Botany*, *114*, pp.117-128.

Jia, X., Meng, Q., Zeng, H., Wang, W. and Yin, H., 2016. Chitosan oligosaccharide induces resistance to Tobacco mosaic virus in *Arabidopsis* via the salicylic acid-mediated signalling pathway. *Scientific Reports*, *6*, p.26144.

Jones, J.D. and Dangl, J.L., 2006. The plant immune system. *Nature*, *444*(7117), pp.323.

Kenton, P., Mur, L.A., Atzorn, R., Wasternack, C. and Draper, J., 1999. (—)-Jasmonic acid accumulation in tobacco hypersensitive response lesions. *Molecular Plant-Microbe Interactions*, *12*(1), pp.74-78.

Kiba, A., Nakano, M., Ohnishi, K. and Hikichi, Y., 2018. The SEC14 phospholipid transfer protein regulates pathogen-associated molecular pattern-triggered immunity in *Nicotiana benthamiana*. *Plant Physiology and Biochemistry*, *125*, pp.212-218.

Klement, Z., Bozso, Z., Ott, P.G., Kecskes, M.L. and Rudolph, K., 1999. Symptomless resistant response instead of the hypersensitive reaction in tobacco leaves after infiltration of heterologous pathovars of *Pseudomonas syringae. Journal of Phytopathology, 147*(7-8), pp.467-475.

Lam, H.N., Chakravarthy, S., Wei, H.L., BuiNguyen, H., Stodghill, P.V., Collmer, A., Swingle, B.M. and Cartinhour, S.W., 2014. Global analysis of the HrpL regulon in the plant pathogen *Pseudomonas syringae* pv. *tomato* DC3000 reveals new regulon members with diverse functions. *PLoS One*, *9*(8), p.e106115.

Lee, S., Ishiga, Y., Clermont, K. and Mysore, K.S., 2013b. Coronatine inhibits stomatal closure and delays hypersensitive response cell death induced by nonhost bacterial pathogens. *PeerJ*, *1*, pp.e34.

Lee, S., Yang, D.S., Uppalapati, S.R., Sumner, L.W. and Mysore, K.S., 2013a. Suppression of plant defense responses by extracellular metabolites from *Pseudomonas syringae* pv. *tabaci* in *Nicotiana benthamiana. BMC Plant Biology*, *13*(1), pp.65.

Lo, T., Koulena, N., Seto, D., Guttman, D.S. and Desveaux, D., 2017. The HopF family of *Pseudomonas syringae* type III secreted effectors. *Molecular Plant Pathology*, *18*(3), pp.457-468.

Marutani, M., Taguchi, F., Ogawa, Y., Hossain, M.M., Inagaki, Y., Toyoda, K., Shiraishi, T. and Ichinose, Y., 2008. *Gac* two-component system in *Pseudomonas syringae* pv. *tabaci* is required for virulence but not for hypersensitive reaction. *Molecular Genetics and Genomics*, *279*(4), p.313.

Misas-Villamil, J.C., Kolodziejek, I. and van der Hoorn, R.A., 2011. *Pseudomonas syringae* colonizes distant tissues in *Nicotiana benthamiana* through xylem vessels. *The Plant Journal*, *67*(5), pp.774-782.

Mittler, R., Simon, L. and Lam, E., 1997. Pathogen-induced programmed cell death in tobacco. *Journal of Cell Science*, *110*(11), pp.1333-1344.

Mur, L.A., Brown, I.R., Darby, R.M., Bestwick, C.S., Bi, Y.M., Mansfield, J.W. and Draper, J., 2000. A loss of resistance to avirulent bacterial pathogens in tobacco is associated with the attenuation of a salicylic acid-potentiated oxidative burst. *The Plant Journal*, *23*(5), pp.609-621.

Mur, L.A., Naylor, G., Warner, S.A., Sugars, J.M., White, R.F. and Draper, J., 1996. Salicylic acid potentiates defence gene expression in tissue exhibiting acquired resistance to pathogen attack. *The Plant Journal*, *9*(4), pp.559-571.

Nomura, K., Melotto, M. and He, S.Y., 2005. Suppression of host defense in compatible plant–*Pseudomonas syringae* interactions. *Current Opinion in Plant Biology*, *8*(4), pp.361-368.

Ortiz-Martín, I., Thwaites, R., Macho, A.P., Mansfield, J.W. and Beuzón, C.R., 2010a. Positive regulation of the Hrp type III secretion system in *Pseudomonas syringae* pv. *phaseolicola*. *Molecular Plant-Microbe Interactions*, *23*(5), pp.665-681.

Ortiz-Martín, I., Thwaites, R., Mansfield, J.W. and Beuzón, C.R., 2010. Negative regulation of the Hrp type III secretion system in *Pseudomonas syringae* pv. *phaseolicola. Molecular Plant-Microbe Interactions*, *23*(5), pp.682-701.

Romantschuk, M., Roine, E. and Taira, S., 2001. Hrp pilus–reaching through the plant cell wall. *European Journal of Plant Pathology*, *107*(2), pp.153-160.

Salch, Y.P. and Shaw, P.D., 1988. Isolation and characterization of pathogenicity genes of *Pseudomonas syringae* pv. *tabaci. Journal of Bacteriology*, *170*(6), pp.2584-2591.

Smirnova, A.V., Wang, L., Rohde, B., Budde, I., Weingart, H. and Ullrich, M.S., 2002. Control of temperature-responsive synthesis of the phytotoxin coronatine in *Pseudomonas syringae* by the unconventional two-component system CorRPS. *Journal of Molecular Microbiology and Biotechnology*, *4*(3), pp.191-196.

Staskawicz, B.J., Dahlbeck, D. and Keen, N.T., 1984. Cloned avirulence gene of *Pseudomonas syringae* pv. *glycinea* determines race-specific incompatibility on *Glycine max* (L.) Merr. *Proceedings of the National Academy of Sciences*, *81*(19), pp.6024-6028.

Studholme, D.J., Ibanez, S.G., MacLean, D., Dangl, J.L., Chang, J.H. and Rathjen, J.P., 2009. A draft genome sequence and functional screen reveals the repertoire of type III secreted proteins of *Pseudomonas syringae* pathovar *tabaci* 11528. *BMC Genomics*, *10*(1), pp.395.

Taylor, J.D., Teverson, D.M., Allen, D.J. and Pastor-Corrales, M.A., 1996. Identification and origin of races of *Pseudomonas syringae* pv. *phaseolicola* from Africa and other bean growing areas. *Plant Pathology*, *45*(3), pp.469-478.

Thwaites, R., Spanu, P.D., Panopoulos, N.J., Stevens, C. and Mansfield, J.W., 2004. Transcriptional regulation of components of the type III secretion system and effectors in *Pseudomonas syringae* pv. *phaseolicola. Molecular Plant-Microbe Interactions*, *17*(11), pp.1250-1258.

Tsunemi, K., Taguchi, F., Marutani, M., WATANABE-SUGIMOTO, M., Inagaki, Y., Toyoda, K., Shiraishi, T. and Ichinose, Y., 2011. Degeneration of *hrpZ* gene in *Pseudomonas syringae* pv. *tabaci* to evade tobacco defence: an arms race between tobacco and its bacterial pathogen. *Molecular Plant Pathology*, *12*(7), pp.709-714.

Turner, J.G. and Debbage, J.M., 1982. Tabtoxin-induced symptoms are associated with the accumulation of ammonia formed during photorespiration. *Physiological Plant Pathology*, *20*(2), pp.223-233.

Turner, J.G. and Taha, R.R., 1984. Contribution of tabtoxin to the pathogenicity of *Pseudomonas syringae* pv. *tabaci. Physiological Plant Pathology*, *25*(1), pp.55-69.

Turner, J.G., 1988. Inhibition of photosynthesis in *Nicotiana tabacum* leaves treated with tabtoxin and its relation to pigment loss. *Physiologia Plantarum*, *74*(3), pp.549-555.

Van Wees, S.C. and Glazebrook, J., 2003. Loss of non-host resistance of *Arabidopsis NahG* to *Pseudomonas syringae* pv. *phaseolicola* is due to degradation products of salicylic acid. *The Plant Journal*, *33*(4), pp.733-742.

Vencato, M., Tian, F., Alfano, J.R., Buell, C.R., Cartinhour, S., DeClerck, G.A., Guttman, D.S., Stavrinides, J., Joardar, V., Lindeberg, M. and Bronstein, P.A., 2006. Bioinformatics-enabled identification of the HrpL regulon and type III secretion system effector proteins of *Pseudomonas syringae* pv. *phaseolicola* 1448A. *Molecular Plant-Microbe Interactions*, *19*(11), pp.1193-1206.

Warner, S.A., Scott, R. and Draper, J., 1992. Characterisation of a wound-induced transcript from the monocot asparagus that shares similarity with a class of intracellular pathogenesis-related (PR) proteins. *Plant Molecular Biology*, *19*(4), pp.555-561.

Warner, S.A., Scott, R. and Draper, J., 1993. Isolation of an asparagus intracellular PR gene (AoPR1) wound-responsive promoter by the inverse polymerase chain reaction and its characterization in transgenic tobacco. *The Plant Journal*, *3*(2), pp.191-201.

Wickham, H., 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer.

Xin, X.F., Kvitko, B. and He, S.Y., 2018. *Pseudomonas syringae*: what it takes to be a pathogen. *Nature Reviews Microbiology*, *16*(5), pp.316.

Zheng, X.Y., Spivey, N.W., Zeng, W., Liu, P.P., Fu, Z.Q., Klessig, D.F., He, S.Y. and Dong, X., 2012. Coronatine promotes *Pseudomonas syringae* virulence in plants by activating a signaling cascade that inhibits salicylic acid accumulation. *Cell Host and Microbe*, *11*(6), pp.587-596.

Zipfel, C., 2014. Plant pattern-recognition receptors. *Trends in Immunology*, *35*(7), pp.345-351.