The Expression of the Antimicrobial Peptide RLK-SMP24 in *Nicotiana benthamiana* and its Localisation to the Plasma Membrane to Prevent Tomato Blight.

Dan Jackson 19066745@brookes.ac.uk

The Plant Pathogen *Phytophthora* and its impact on the *Solanaceae* Family

Phytophthora infestans is a plant pathogen that is responsible for tomato blight and the 1845-49 Irish famine [1;2]. *P. infestans* infects members of the *Solanaceae* family, which includes potatoes, tomatoes and peppers, and destroys the food they produce (Figure 1)[3]. Infection by *P. infestans* is achieved through the use of haustorium (Figure 2).

Figure 1. The effect of *P. infestans* on potato tubers (Left) and tomato fruit (Right).

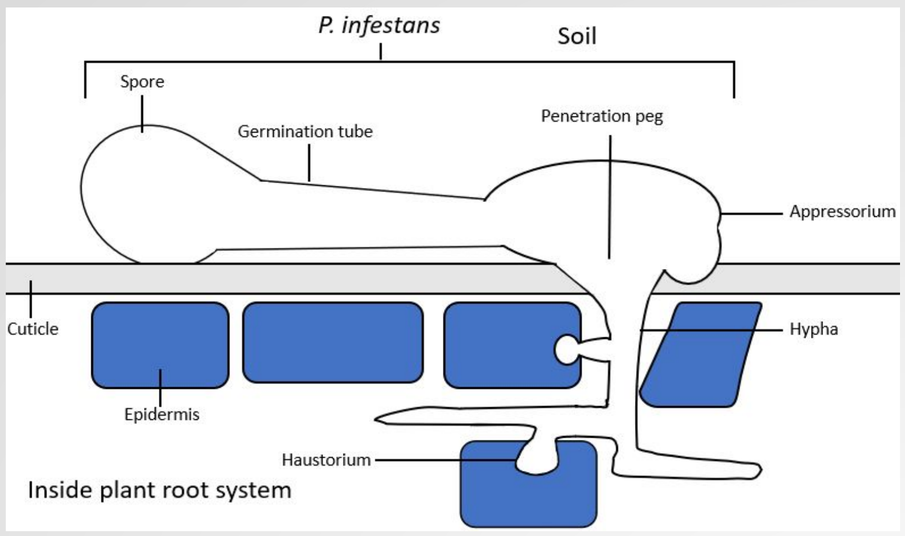


Figure 2. Illustration of how *P. infestans* infects members of the Solanaceae family using haustoria to invade cells and modulate cellular processes (Adapted from [4]).

The use of Antimicrobial Peptides (AMPs) against *P. infestans*

The agricultural industry overuses fungicides to prevent crop damage by *P. infestans* [5;6;7]. Fungicides negatively impact the environment and *P. infestans* has developed resistance to the fungicides used against it [8;9]. Antimicrobial Peptides (AMPs) from scorpion and snake venom are biodegradable, known to avoid resistance and have been shown to inhibit *P. infestans* growth. Because of this, AMPs from snake and scorpion venom could be a novel treatment to *P. infestans* [10].

Use of *N. benthamiana* as a model organism

Nicotiana benthamiana is widely used for studying plant-pathogen interactions and, like potatoes and tomatoes, *N. benthamiana* is a member of the Solanaceae family [11;12]. This means that the findings of this project should be transferable to potatoes, tomatoes and peppers.

Aims and Objectives

This project aims to express an AMP from scorpion venom, SMP24, in *N. benthamiana* as a model system to fight blight (Figure 3). The peptide will be targeted to the plasma membrane by fusing it to a receptor-like kinase (RLK). This localisation outside the plasma membrane could benefit the functionality of SMP24 and should prevent *P. infestans* haustorium from entering the cell [1;13;14].

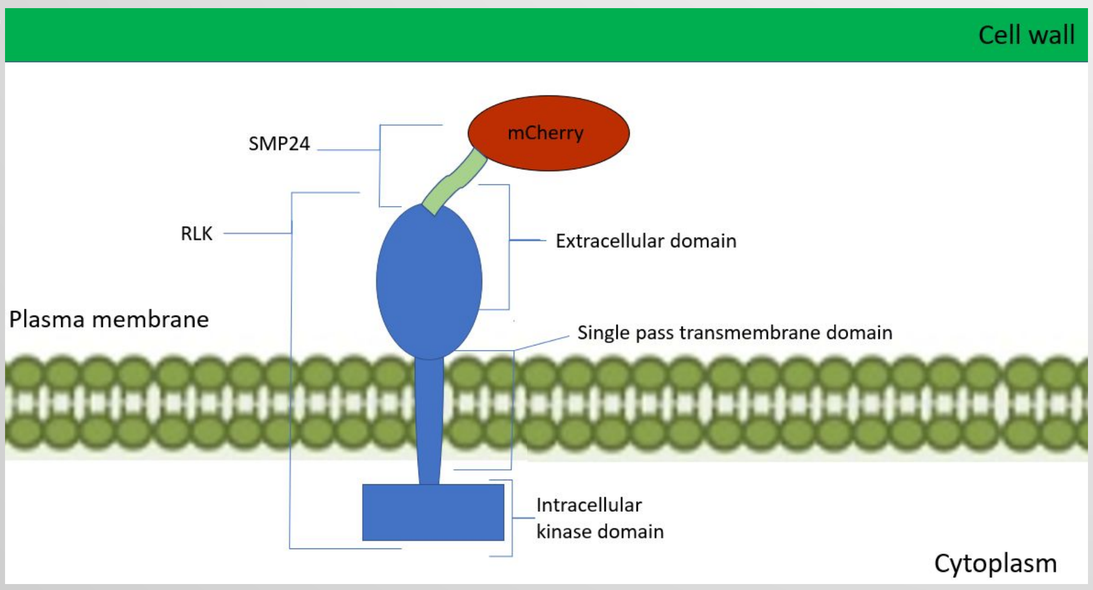


Figure 3. Illustration of the RLK-SMP24 fusion protein, with the red fluorescent protein mCherry attached, in the plasma membrane of a plant cell with the extracellular domain facing towards the apoplast (Adapted from: [15;16]).

Methodology

The fusion protein RLK-SMP24 was cloned into a plant expression vector using gateway cloning (Figure 3;4).

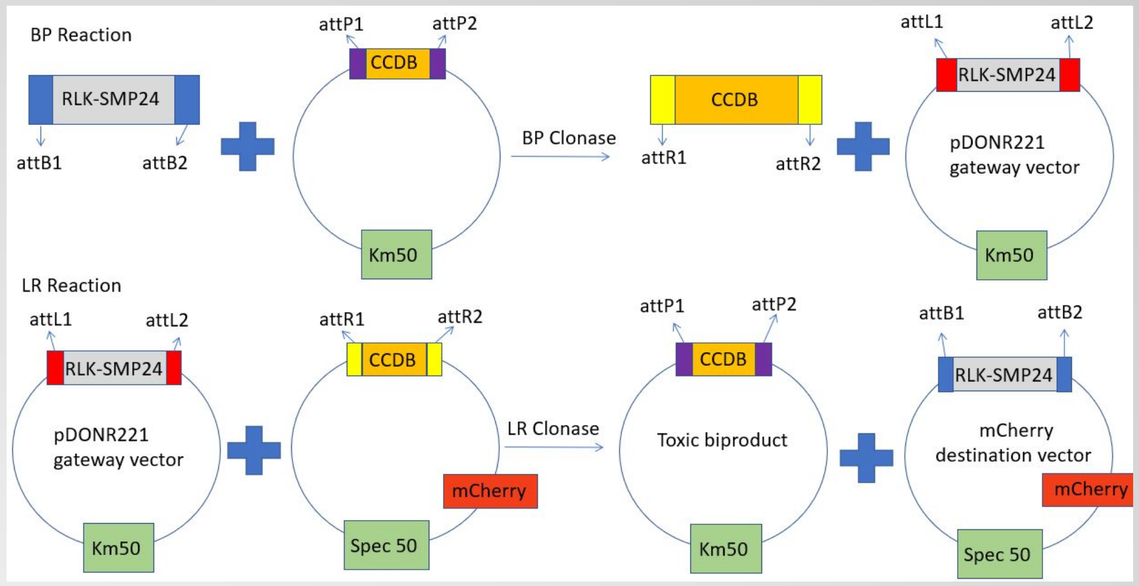


Figure 4. Overview of the two steps, the BP and LR reactions, of Gateway cloning and the vectors produced.

The RLK-SMP24-mCherry destination vector was then inserted into the plant pathogen *Agrobacterium tumefaciens* and infiltrated into *N. benthamiana* leaf cells (Figure 5). Laser confocal microscopy was used to see if the AMP was being expressed, that the expression was targeted to the plasma membrane only, and to assess the effect of the AMP on plant health.

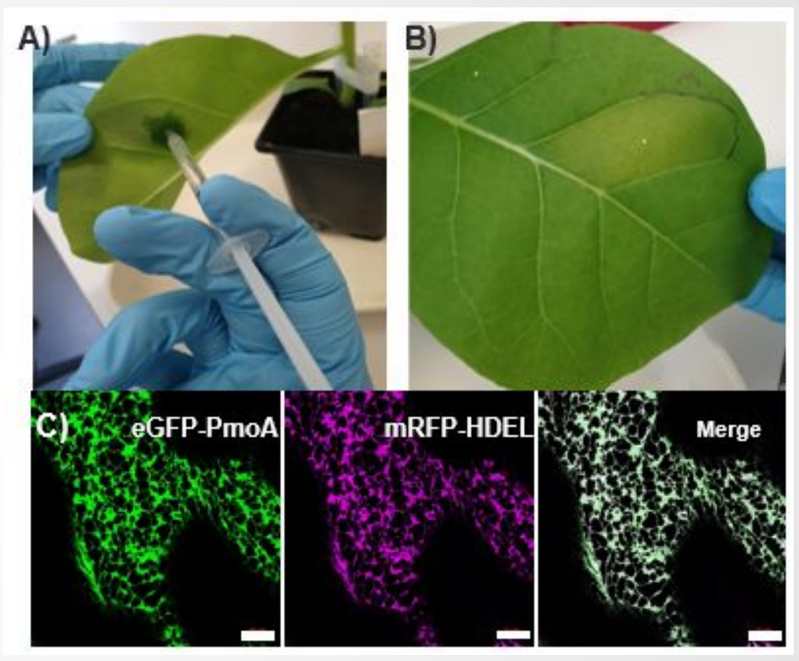
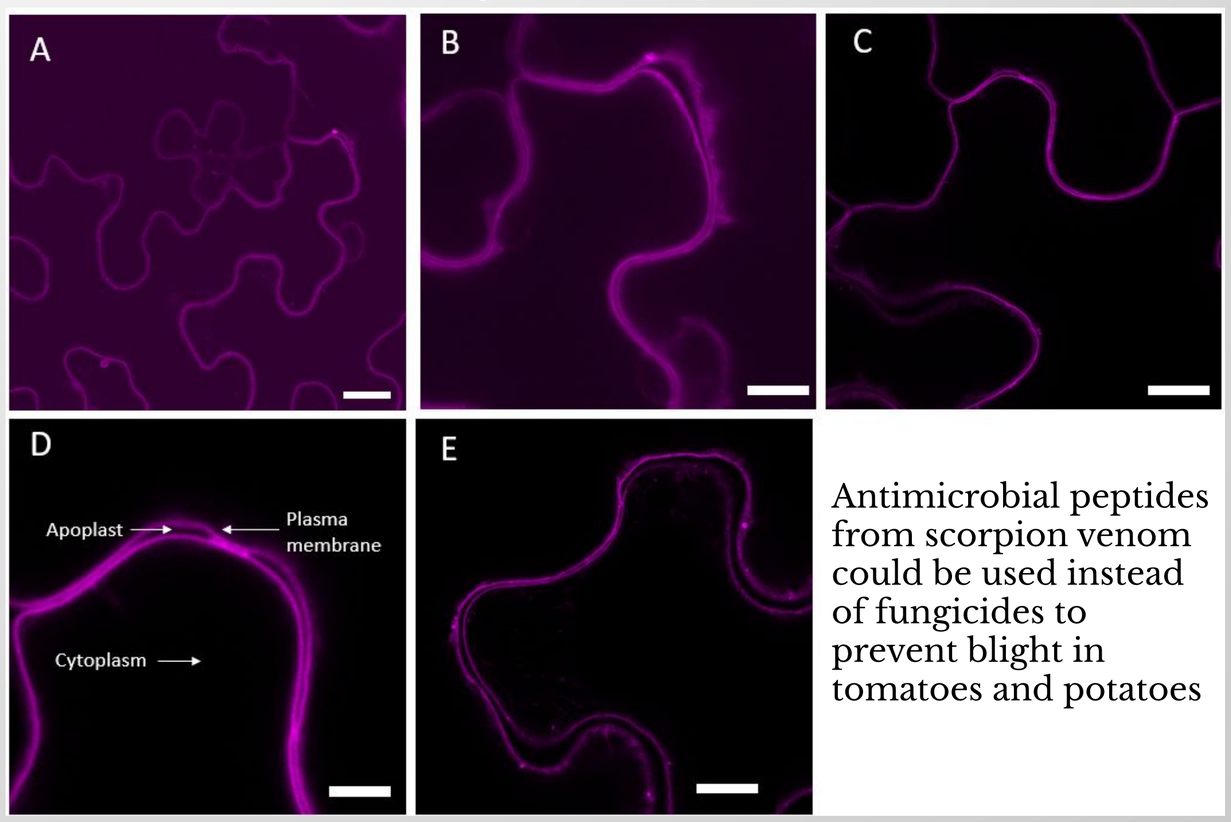


Figure 5. Tobacco leaf infiltration
A) The Agrobacterium suspension in infiltrated into the leaf from the lower leaf side using a 1 ml syringe.
B) The area filled with the Agrobacterium suspension is marked with a permanent pen.
C) Example confocal image for localised PmoA protein expression (green).
Correct localisation is shown by overlap with the ER marker RFP-HDEL (magenta). Size bars = 10 µm.

Results

Confocal microscopy (Figure 6) showed that RLK-SMP24-mCherry was expressed and localised to the plasma membrane as predicted. Cellular structures look intact, showing no effect on plant health.



Antimicrobial peptides from scorpion venom could be used instead of fungicides to prevent blight in tomatoes and potatoes

Figure 6. Confocal microscopy images showing RLK-SMP24 expression and localisation to the plasma membrane of *N. benthamiana* leaf cells. Size bars = 10 µm for A, 5 µm for B and C and 2 µm for D and E.

Discussion

This study shows that the RLK-SMP24 can be expressed and targeted in *N. benthamiana*. This can be applied to future research into antimicrobial peptides and their effect on other plant pathogens, to express antimicrobial peptides in other plants or as an alternative to currently used fungicides. Future work will involve the creation of plants expressing the antimicrobial peptide in a stable manner and to test its functionality against *P. infestans*.

References

1. Ristaino, J. B. (2002) Tracking historic migrations of the Irish potato famine pathogen, *Phytophthora infestans*. *Microbes and Infection*, **4**(13), 1369-1377.
2. Rossman, A. Y. (2008) The impact of invasive fungi on agricultural ecosystems in the United States. In *Ecological Impacts of Non-Native Invertebrates and Fungi on Terrestrial Ecosystems* (97-107). Springer, Dordrecht.
3. Murphy, F., He, Q., Armstrong, M., Giuliani, L. M., Boevink, P. C., Zhang, W., Tian, Z., Birch, P. and Gilroy, E. M. (2018) The potato MAP3K StVIK is required for the *Phytophthora infestans* RXLR effector Pi17316 to promote disease. *Plant Physiology*, **177**(1), 398–410.
4. Meng, S., Torto-Alalibo, T., Chibucos, M.C., Tyler, B.M. and Dean, R.A. (2009) Common processes in pathogenesis by fungal and oomycete plant pathogens, described with Gene Ontology terms. *BMC Microbiology*, **9**, 1-11.
5. Naveed, Z. A., Wei, X., Chen, J., Mubeen, H. and Ali, G. S. (2020) The PTI to ETI continuum in Phytophthora-plant interactions. *Frontiers in Plant Science*, **11**, 593905.
6. Fabritius, A. L., Shattock, R. C. and Judelson, H. S. (1997) Genetic analysis of metalaxyl insensitivity loci in *Phytophthora infestans* using linked DNA markers. *Phytopathology*, **87**(10), 1034–1040.
7. Judelson, H. S. and Roberts, S. (1999) Multiple loci determining insensitivity to phenylamide fungicides in *Phytophthora infestans*. *Phytopathology*, **89**(9), 754-760.
8. Maróti, G., Kereszt, A., Kondorosi, É. and Mergaert, P. (2011) Natural roles of antimicrobial peptides in microbes, plants and animals. *Research in Microbiology*, **162**(4), 363-374.
9. Holaskova, E., Galuszka, P., Frebort, I. and Oz, M. T. (2015) Antimicrobial peptide production and plant-based expression systems for medical and agricultural biotechnology. *Biotechnology Advances*, **33**(6), 1005-1023.
10. Zhou, Y., Yang, K., Yan, Q., Wang, X., Cheng, M., Si, J., Xue, X., Shen, D., Jing, M., Tyler, B. M. and Dou, D. (2021) Targeting of anti-microbial proteins to the hyphal surface amplifies protection of crop plants against Phytophthora pathogens. *Molecular Plant*, **14**(8), 1391–1403.
11. Goodin, M. M., Zaitlin, D., Naidu, R. A. and Lommel, S. A. (2008) *Nicotiana benthamiana*: its history and future as a model for plant–pathogen interactions. *Molecular Plant-Microbe Interactions*, **21**(8), 1015-1026.
12. Huang, J., Lu, X., Wu, H., Xie, Y., Peng, Q., Gu, L., Wu, J., Wang, Y., Reddy, A. and Dong, S. (2020) Phytophthora effectors modulate genome-wide alternative splicing of host mRNAs to reprogram plant immunity. *Molecular Plant*, **13**(10), 1470–1484.
13. Toth, I. K. and Birch, P. R. (2005) Rotting softly and stealthily. *Current Opinion in Plant Biology*, **8**(4), 424–429.
14. Hématy, K., Cherk, C. and Somerville, S. (2009) Host–pathogen warfare at the plant cell wall. *Current Opinion in Plant Biology*, **12**(4), 406-413.
15. Macho, A. P and Lozano-Duran, R. (2019) Molecular dialogues between viruses and receptor-like kinases in plants. *Molecular Plant Pathology*, **20**(9), 1191-1195.
16. Elrayess, R. A., Mohallal, M. E., El-Shahat, Y. M., Ebaid, H. M., Miller, K., Strong, P. N., & Abdel-Rahman, M. A. (2020) Cytotoxic effects of Smp24 and Smp43 scorpion venom antimicrobial peptides on tumour and non-tumour cell lines. *International Journal of Peptide Research and Therapeutics*, **26**(3), 1409-1415.