

# Functional characterization of the RxLR-EER translocation signal for delivery of oomycete effector proteins into host plant cells



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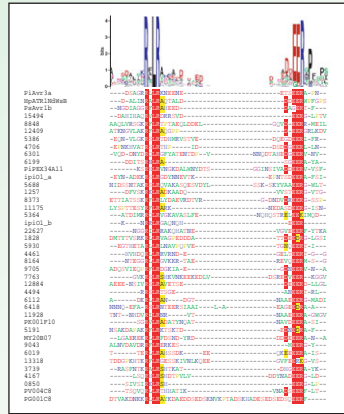
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## Background

Avirulence genes encode proteins recognised by resistant host plants, triggering hypersensitive cell death and containment of infection. Oomycete avirulence proteins possess a common dual motif, RxLR-EER, located near the N-terminus of the mature proteins.<sup>1</sup> Cognate host resistance proteins are predicted to be cytoplasmic. The oomycete RxLR-EER was shown to be functionally analogous to the host targeting signal RxLxE/D/Q in malaria parasites:<sup>2</sup> RxLR-EER function in oomycetes is not yet demonstrated.

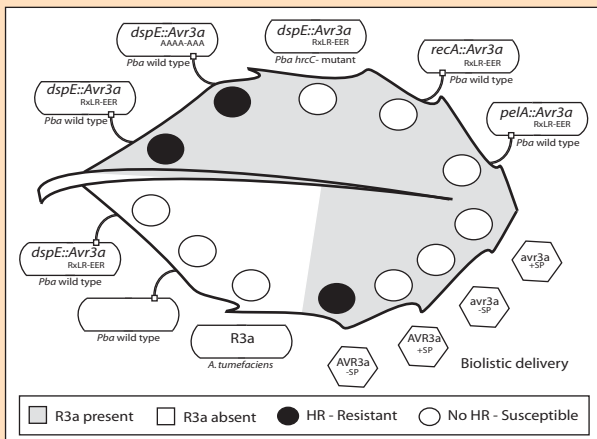


RxLR-EER sequence logo of alignment shown below for 3 oomycete Avr and 38 expressed *P. infestans* proteins. For alignment, sequences were N- and C-terminal trimmed.

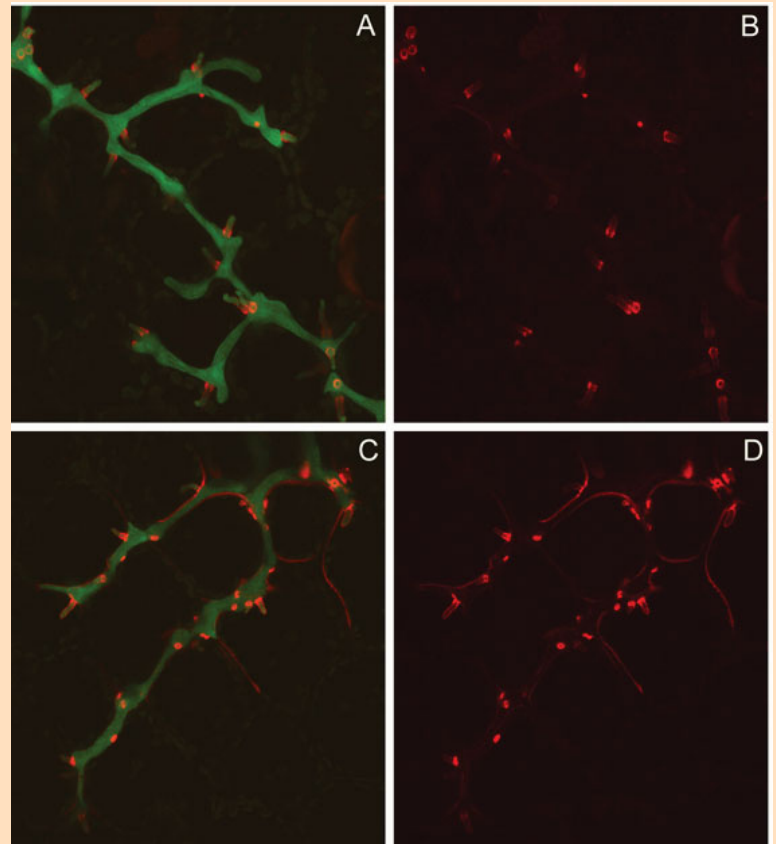
## Strategy

The avirulence gene *Avr3a* from the potato late blight pathogen *Phytophthora infestans* was used as a reporter for RxLR-EER delivery as it yields a clear phenotype on *R3a* potato plants. AVR3a was delivered into plant cells or the plant apoplast using the T2SS or T3SS of the bacterial potato pathogen, *Pectobacterium atrosepticum*. RxLR, EER, or both, in AVR3a were replaced by alanine residues in stably transformed *P. infestans*. The RxLR-EER motif in AVR3a is not required for recognition by *R3a*.<sup>3</sup> Transformants were assayed for recognition and resistance on *R3a* potato plants. Native AVR3a and alanine replacement constructs were fused to mRFP in stable *P. infestans* transformants for localisation during infection of potato using confocal microscopy.

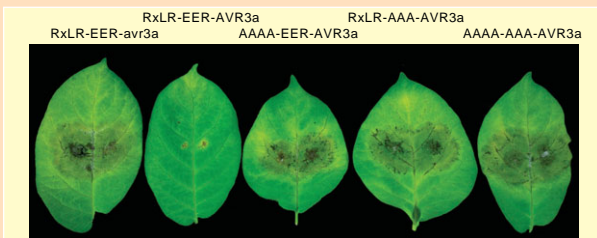
## Results



AVR3a is recognised inside plant cells. RxLR-EER effector AVR3a can be translocated, via the T3SS (*dspE*::AVR3a) directly into the host cell from the wild type bacterial plant pathogen, *P. atrosepticum* (*Pba*). When co-infiltrated with *Agrobacterium tumefaciens* delivering *R3a* the HR is triggered. When AVR3a is fused to a non-secreted protein (*recA*) in *Pba*, or the T2SS *pelA* protein (secreted into plant intercellular spaces), there is no HR. With the T3SS disabled (*hrcC*- mutant) AVR3a is no longer translocated inside the plant cell (no HR). Biolistic delivery of constructs encoding 35S::AVR3a minus a signal peptide (SP) for secretion results in HR; AVR3a plus SP, *avr3a* plus/minus SP do not result in HR.



*P. infestans* transformants expressing cytoplasmic eGFP and *Avr3a*::mRFP translational fusions. (A) AVR3a::mRFP in infected potato leaf tissue showing secretion of AVR3a specifically from finger-like haustoria. (B) red only image of same field as in A. (C) AVR3a::mRFP with RxLR-EER replaced by alanine residues in infected potato leaf tissue. Red fluorescence was observed at haustoria and also in the host apoplast adjoining the haustoria. (D) red channel image of same field as in C, showing extent of red fluorescence leakage from haustoria in alanine replacement transformants.



Reactions of potato cv. Pentland Ace (*R3a*) to alleles and ala replacements of *Avr3a* transformed into an *avr3a* isolate

## Conclusions

AVR3a cannot passively enter host cells from the apoplast; it requires a specific environment or mechanism to cross the plant cell membrane. Translocation of AVR3a into host cells is dependent on both RxLR and EER motifs. AVR3a is secreted from finger-shaped biotrophic haustoria. Alanine replacement AVR3a::mRFP fusion constructs accumulate and overspill the extra-haustorial matrix into the apoplast.

## References

- 1 Rehmany et al. 2005 Plant Cell 17:1839-50.
- 2 Bhattacharjee et al. 2006 PLoS Pathogens DOI:10.1371/journal.ppat.0020050.
- 3 Bos et al. 2006 Plant J. 48:165-76.

## Future research

Conservative changes in the RxLR-EER of AVR3a to reveal specificity of translocation mechanism. Visualisation of AVR3a translocation into host cells. RxLR domain swaps between RxLR effector proteins from different oomycetes.

## Acknowledgements

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