# Expression profiles of RXLR motif-containing sequences during Phytophthora infestans-potato interactions

Juan Morales1, Anna Avrova1, Carolyn Young2, Steve Whisson1, Leighton Pritchard1, Sophien Kamoun2, Paul Birch1.

1 SCOTTISH CROP RESEARCH INSTITUTE, Invergowrie, Dundee, UK. DD2 5DA.

2 THE OHIO STATE UNIVERSITY, Department of Plant Pathology, Selby Hall 212, 1680 Madison Avenue, Wooster Ohio 44691-4096.

Contact e-mail: juan.morales@scri.ac.uk, paul.birch@scri.ac.uk

























### Introduction

Phytophthora infestans is the causal agent of potato and tomato late blight disease which causes losses on a world wide basis of more than US\$5 billion annually (Duncan J. 1999. Microbiology Today. 26:114-116.). All cloned Oomycete avirulence genes share the RXLR motif (Rehmany et al., 2005. The Plant Cell. 17:1839-1850.). The role of this motif is unknown but, based on a similar motif found in secreted proteins from *Plasmodium*, the malaria parasite, a role in translocating proteins into the host cytoplasm can be speculated. A detailed bioinformatics search revealed approximately 50 P. infestans sequences sharing the RXLR motif.



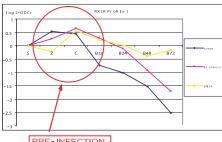
## **Objectives**

- 1. To determine gene expression profiles of RXLR motif-containing sequences through preinfection and infection of potato plants (cv. Bintie) by P. infestans isolate 88069
- 2. To examine variability on effector gene expression among different isolates

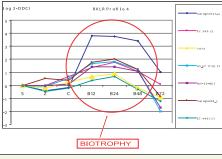
### **Methods**

Real-time reverse transcription polymerase chain reaction (RT-RT-PCR) analysis was used to quantify relative gene expression levels in different *P. infestans* (isolate 88069) stages of development: sporangia (S), zoospores (Z), cyst (C), and during infection (12, 24, 48 and 72 hours post inoculation (h.p.i.) on susceptible potato cv. Bintje. Actin A was used as a constitutively expressed endogenous reference (Avrova et al., 2003. Fungal Genetics and Biology. 40:4-14.). For comparison, 2 non-RXLR effector genes have been included in this analysis: Epic 3 and Epic4.

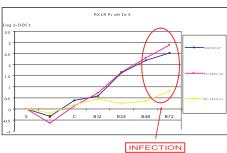
### Results







# RXLR profile 2 PRE-INFECTION AND BIOTROPHY



Gene expression analysis of RXLR motif-containing sequences: grouping the gene expression of 49 RXLR motif-containing sequences, five profiles were determined with genes up-regulated during different stages of pre-infection and infection.

### Conclusions

sequences.

All RXLR motif-containing sequences are up-regulated in preinfection or infection stage. These genes are good candidates to be P. infestans effectors.

Avr3a paralogue Pex147-2 is up-regulated during infection in five out of nine isolates evaluated.

Avr3a paralogue Pex 147-3 is only detectable at 72 hpi expressed at 72 hpi in isolates Ca65 and Sc95 17.4.2, but down regulated relative to sporangia. The putative cysteine protease inhibitors Epic4 (profile 1) and

Epic3 (profile 2) are co-expressed with RXLR motif-containing

Avr3a gene expression analysis on a range of isolates showed up-

Previously reported genes matched some profiles, Avr3a Armstrong et al., 2005. PNAS. 102(21):7766-7771) (profile 3) and IpiO (Van West P. et al., 1998. Fungal Genetics and Biology.

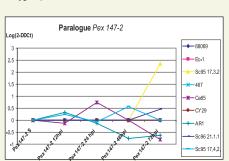
regulation in most of them during in-planta infection (8 out of 9).

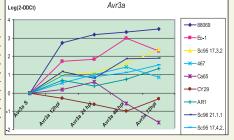
23:126–138) (profile 2), addressing the importance of further studies of RXLR motif-containing sequences as possible P. infestans effectors.

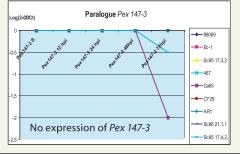
### Variation in effector gene expression

Avr3a has two paralogues, Pex147-2 and Pex 147-3 (Armstrong et al., 2005. PNAS. 102(21):7766-7771). To investigate the possible role that variation in gene expression and paralogue genes play in *P. infestans*-potato interactions, gene expression analysis was performed on a range of isolates differing in the Avr3a alleles (avirulent homozygous and heterozygous and virulenthomozygous).









### **Future Research**

Silencing and functional studies are needed to investigate the role of RXLR motif-containing sequences in late blight disease.

To investigate the molecular interactions of AVR3a in the host cytoplasm to elucidate its role in potato pathogenicity.

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