

Screening potato genotypes for responses to RxLR class effector genes from *Phytophthora infestans*



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INTRODUCTION

Phytophthora infestans causes late blight disease of potato and tomato. All cloned oomycete avirulence genes share an RxLR motif, suggested to have a role in translocating proteins into the host cytoplasm. All but one also share an additional EER motif downstream of the RxLR. A detailed bioinformatics search revealed 425 potential *P. infestans* sequences sharing the RxLR and EER motifs. Gene expression and functional analysis has been carried out for this class of effectors.



OBJECTIVES

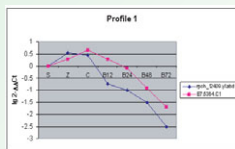
1. To determine gene expression profiles of RxLR class effectors through pre-infection and infection of potato plants (cv. Bintje) by *P. infestans* isolate 88069.
2. To investigate whether they trigger responses in plants using a PVX binary vector, *Agrobacterium tumefaciens* infiltration and biolistics.



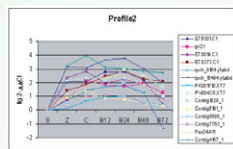
GENE EXPRESSION PROFILES OF RxLR CLASS EFFECTORS

MATERIALS AND METHODS

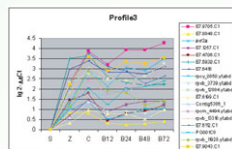
Reverse Transcription Real-time polymerase chain reaction (RT-RT-PCR) analysis was used to quantify relative gene expression levels in different *P. infestans* (isolate 88069) stages of development: sporangia (S), zoospores (Z), germinating cysts (C), and during infection (12, 24, 48 and 72 hours post inoculation (h.p.i.) on susceptible potato cv. Bintje. Actin A was used as a constitutively expressed endogenous reference.



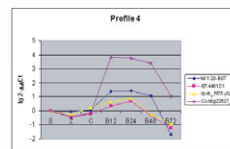
Pre-infection



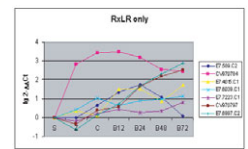
Pre-infection and throughout infection with expression levels dropping during necrotrophy



Pre-infection and throughout infection



Biotrophy only



Grouping of seven genes sharing RxLR motif only

Grouping the gene expression of 38 RxLR-EER class effectors identified four profiles with genes up-regulated during different stages of pre-infection and infection.

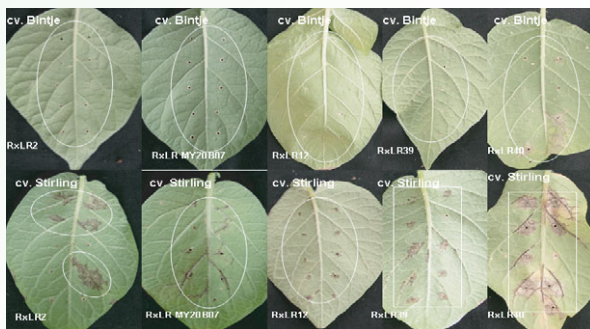


FUNCTIONAL ANALYSIS OF RxLR CLASS EFFECTORS

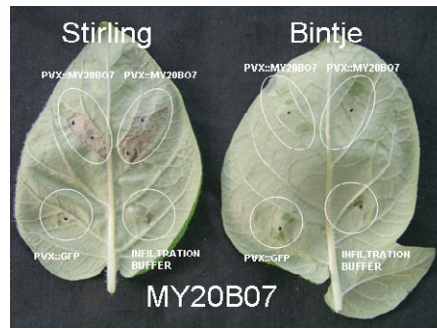
MATERIALS AND METHODS

The ability of RxLR class effectors to trigger cell death in susceptible potato cv. Bintje and in field resistant cv. Stirling was carried out using expression from a potato virus X (PVX)-based vector, *Agrobacterium tumefaciens* (agro-infiltration), and a biolistics approach for cobombardment with the beta-glucuronidase (GUS) gene.

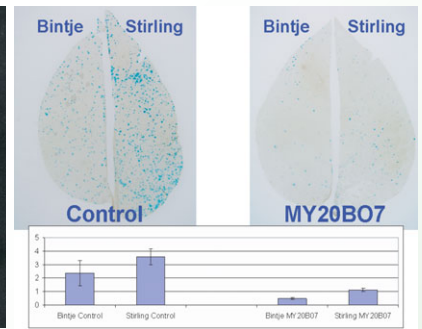
PVX toothpick inoculations



PVX Agro-Infiltration

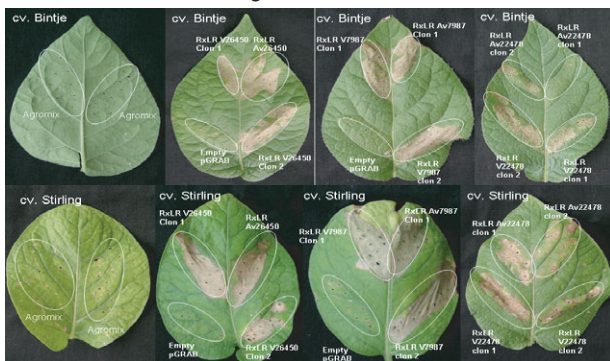


Co-bombardment of MY20B07



Five different RxLR class effector genes caused a cell death response in plants of the field resistant potato cv. Stirling when expressed from the PVX-based vector. When co-bombarded with the GUS gene, RxLR MY20B07 showed a significant reduction in cell vitality compared with the controls.

Agro-infiltration



Three RxLR effectors induced cell death after infiltration in both cv. Bintje and cv. Stirling



CONCLUSIONS

All RxLR class genes analyzed are up-regulated in pre-infection or infection stages.

RxLR effectors MY20B07, 26450, 7987 and 22478 induce cell death in susceptible cv. Bintje and in field resistant cv. Stirling by Agro-infiltration.

Some RxLR class effectors may promote cell death when overexpressed in potato cells.