


Identification of effectors involved in the *Leptosphaeria maculans*-canola interaction

Alexander Idnurm
School of BioSciences
University of Melbourne



Research performed and supported by

Candace Elliott
Barbara Howlett
Rohan Lowe
Kerryn Popa
Andrew Urquhart
Angela Van de Wouw

Jessica Chung
Jacqui Batley and her team



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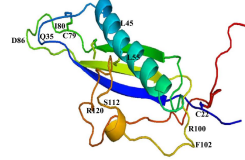
Blackleg disease management involves effector/R gene interactions



%*Sylvestris*+*Rlm1* lines destroyed by blackleg (November 2004)

General characteristics of effectors

- ~ Small secreted proteins.
- ~ Rich in cysteine amino acid residue.
- ~ Highly expressed during plant disease.
- ~ Little or no sequence similarity between them.
- ~ Difficult to detect in fungal genomes, especially in *L. maculans* in which they usually are found within long stretches of repetitive DNA.
 - ~ *AvrLm1, AvrLm2, AvrLm3, AvrLm4-7, AvrLmJ1, AvrLm6, AvrLm11* identified.
- ~ Discovery is essential to assist in testing cultivar resistance and pathogen population surveillance.



Blondeau et al. 2015

RNA-seq comparison of transcriptomes

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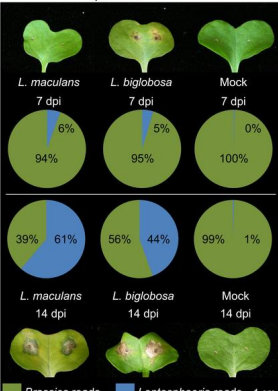
Genomes and Transcriptomes of Part Fungal- Interactions between Canola and Two *Leptosphaeria* Species

Rohan G. T. Lowe¹, Andrew Cassin², Jonathan Grandaubert³, Betha Wouw¹, Thierry Rouxel¹, Barbara J. Howlett^{1*}

PLOS ONE | www.plosone.org

~ The known avirulence genes were all among the most highly upregulated genes early during infection.

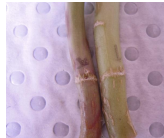
~ Other candidate effectors were also highly upregulated.




Treatment	7 dpi	14 dpi
<i>L. maculans</i>	6% Brassica, 94% Leptosphaeria	39% Brassica, 61% Leptosphaeria
<i>L. biglobosa</i>	5% Brassica, 95% Leptosphaeria	56% Brassica, 44% Leptosphaeria
Mock	0% Brassica, 100% Leptosphaeria	99% Brassica, 1% Leptosphaeria

Stem cankering is a major cause of yield loss: how similar is this process to cotyledon disease?

Mock



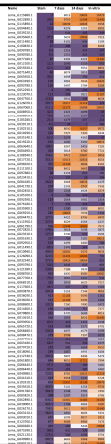
L. maculans



- ~ 110 days after inoculation with *L. maculans*, *L. biglobosa* or water.
- ~ Two RNA samples were extracted and the RNA sequenced.

Summary of RNA-seq analysis from stem tissue

- ~ Different set of regulated genes stems compared to cotyledons in *L. maculans*.
- ~ Highly expressed genes specifically in stems are for:
 - ~ cell wall degrading enzymes.
 - ~ uncharacterised secreted proteins.
- ~ Low expressed genes in stems are:
 - ~ all five avirulence genes known in the genome of strain M1.

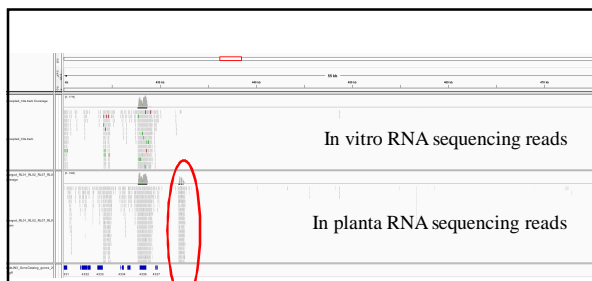


Small secreted proteins at the interface of the *L. maculans*-*B. napus* interaction

- (1) Prediction of unknown effectors based on genome sequencing.
- (2) Known avirulence genes do not function in stem lesion interactions with the host.
- (1) Altered expression of stem-specific+ secreted proteins reduces fungal pathogenicity on cotyledons.

Discovery of unknown effector-encoding genes

- ~ Often missed or incorrectly annotated in the *L. maculans* genome sequence.
- ~ We cannot rely on standard gene prediction methods to identify them.
 - ~ Example: *AvrLm3*
- (1) Scanned the genome sequence for RNA reads that fit the pattern for effectors.
- (2) Computer-based detection.

In vitro RNA sequencing reads

In planta RNA sequencing reads

MRHATIASLFLYAASLCAASI PGNAPGNVGLVERGEQCHKDRYWEWETCY
 NNNSCSRHEKWKCSAQNTANYNKPQDMAKCGADCCRYCHITKESYGVAC

- ~ This manual approach revealed 15 candidate effectors that had not been currently predicted in *L. maculans*.

Discovery of unknown effector-encoding genes

- ~ 12 samples of cotyledons infected with 12 differential strains.
- ~ RNA-sequencing by Jacqui Batley.
- ~ Analysis of data by Jessica Chung at VLSCI.
- ~ De novo assembly of the reads, i.e. a method that does not require a perfect genome sequence.
- ~ 78 candidate effectors.

No gene-for-gene interaction in stem cankers

Actin regulated *AvrLm6*

Actin regulating a control gene

cv. Aurea

Overexpression of stem+genes in cotyledons can reduce pathogenicity

Small secreted proteins highly expressed in the stem but low in the cotyledon were fused to the *AvrLm6* promoter to drive expression during early stages of cotyledon disease.

Control transformant

Expressing a stem-specific SSP

Overexpression of stem+genes in cotyledons can reduce pathogenicity

- Old or senescent cotyledons, when *Avr* genes are likely down-regulated in the fungus, succumb to disease.
- Suggests that there is recognition of fungus early during disease.

Summary

- Gene expression profiles in stem cankers vs. in cotyledons are different, including the arsenal of fungal small secreted proteins that interact with the host.
- Known *Avr* genes highly expressed in the cotyledon and lowly expressed in stem.
- The *Avr/R* system present in cotyledons is absent from the stems.
- The RNA-seq data provides insight into other putative small secreted proteins that may be involved in disease.

