

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

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Australian Government
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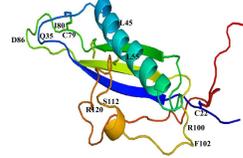
Blackleg disease management involves effector/R gene interactions



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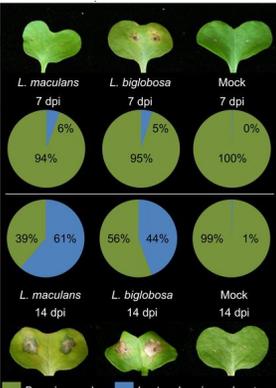
Genomes and Transcriptomes of Part Fungal- Interactions between Canola and Two *Leptosphaeria* Species

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

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

~ Other candidate effectors were also highly upregulated.



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

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.

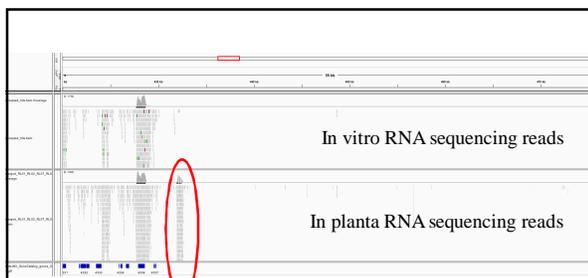


Small secreted proteins at the interface of the *L. maculans*-*B. napus* interaction

- (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.

