


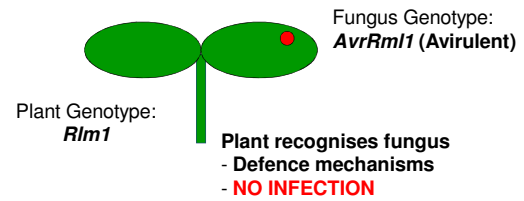
Cloning a gene in the blackleg fungus, *Leptosphaeria maculans* conferring avirulence towards *Brassica juncea*

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Resistance and avirulence genes

- Plant contains resistance genes (R genes)
- Pathogen contains avirulence genes (Avr genes) corresponding to R genes



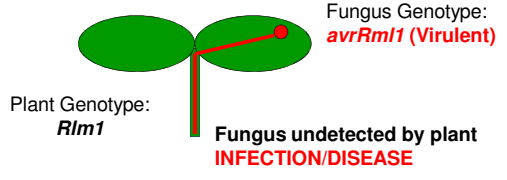
Plant Genotype: **Rlm1**

Fungus Genotype: **AvrRml1 (Avirulent)**

Plant recognises fungus
- Defence mechanisms
- **NO INFECTION**

Resistance and avirulence genes

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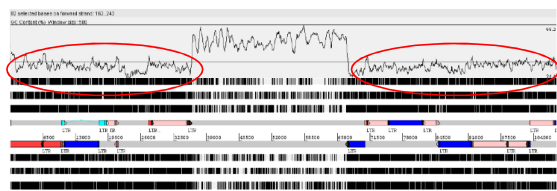
Plant Genotype: **Rlm1**

Fungus Genotype: **avrRml1 (Virulent)**

Fungus undetected by plant
INFECTION/DISEASE

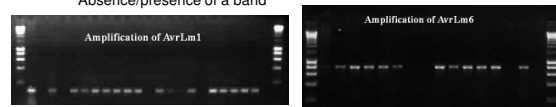
Four avirulence genes have been cloned from *L. maculans*

- AvrLm1 = Rlm1 (AV-Garnet)
- AvrLm4 = Rlm4 (CB-Telfer)
- AvrLm6 = Rlm6 (*B. juncea*)
- AvrLm11 = Rlm11 (*B. rapa*)
 - All located in gene-poor regions of the genome and highly up-regulated in planta

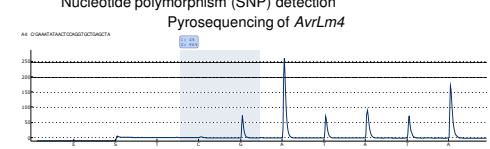


Molecular markers can be developed for avirulence genes

Absence/presence of a band

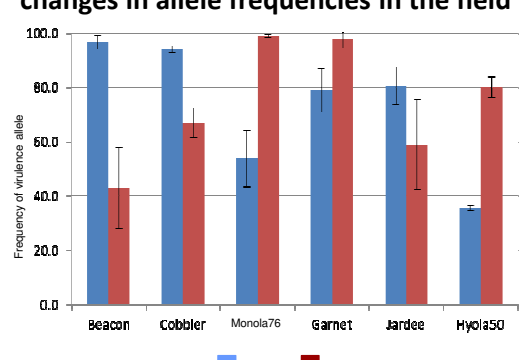


Nucleotide polymorphism (SNP) detection
Pyrosequencing of AvrLm4



Van de Wouw et al (2010) Plant Pathology 59, 809-818
Van de Wouw et al (2010) PLoS Pathogens 6(11)e1001180
Van de Wouw and Howlett (2012) Journal of Applied Microbiology 113, 1145-1153

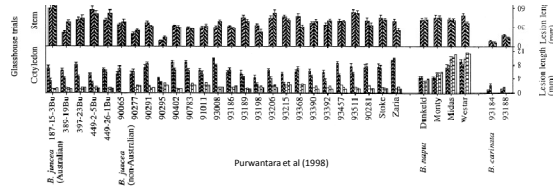
Molecular markers can be used to monitor changes in allele frequencies in the field



Genotype	AvrLm4 Frequency (%)	AvrLm1 Frequency (%)
Beacon	~95	~45
Cobbler	~95	~65
Monola76	~55	~95
Garnet	~65	~95
Jardee	~65	~60
Hyola50	~35	~65

Candidate *B. juncea* avirulence gene

- Previous work in Howlett lab identified juncea-attacking blackleg isolates
 - These isolates attack all 92 *B. juncea* lines screened
 - Virulence towards *B. juncea* segregated as a single gene
 - Species-specific *a/virulence* gene in *L. maculans*



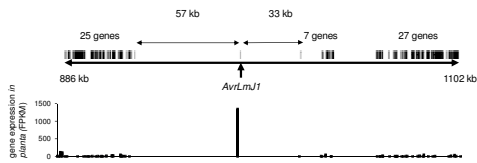
A/Virulence towards juncea mapped onto SuperContig7 of *L. maculans*

- Crosses set up between a juncea-attacking isolate (IBC18) and a non-attacking isolate (04P014).
- Both isolates virulent towards *Rlm6*, a juncea-resistance gene introgressed into *B. napus*.
- 66 progeny were screened for virulence on juncea and with molecular markers
- A/virulence mapped to SC7



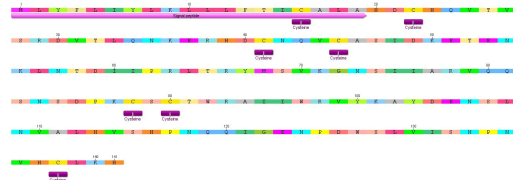
Candidate gene identified using RNAseq

- RNA-seq data from infected cotyledons (7 dpi) and *in vitro* growth used to identify genes highly up-regulated *in planta*.
- Within mapped region on SC7, three SSPs identified
 - two had low expression *in planta*
 - one (LemaT070880) up-regulated 5380 fold *in planta*



LemaT070880 is candidate avirulence gene

- LemaT070880 encodes a small secreted, cysteine rich protein located in AT-rich region
- RNA-seq data showed incorrect gene prediction in reference genome; correct annotation has earlier start codon.
- Gene sequenced from attacking and non-attacking isolates



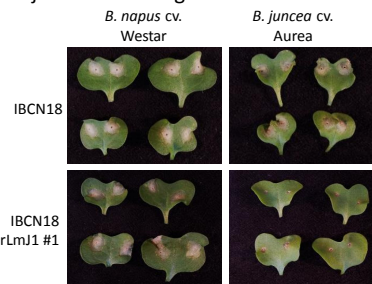
Premature stop-codon in juncea-attacking isolates

- Stop codon identified in juncea-attacking isolates
- Presence of stop codon shows 100% correlation with virulence in progeny

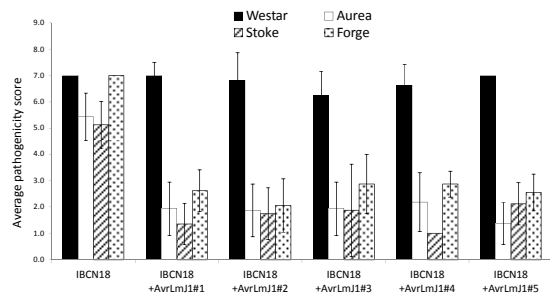
Allele	Isolates (frequency)	# of nucleotide changes	Coding sequence change	Phenotype on <i>B. juncea</i> cultivars
Lema_uP070880_2_0	34 (43%)	N/A	N/A	Avirulent
Lema_uP070880_2_1	18 (23%)	1	K ⁵⁵ R	Avirulent
Lema_uP070880_2_2	15 (19%)	1	K ⁵⁵ T	Avirulent
Lema_uP070880_2_3	9 (12%)	2	R ³⁶ L, K ⁵⁵ R	Avirulent
Lema_uP070880_2_4	2 (3%)	3	R ³⁶ Stop, R ³⁶ L, K ⁵⁵ R	Virulent

LemaT070880 confers avirulence towards Juncea cultivars - I

- Complementation construct made and transformed into juncea-attacking isolate



LemaT070880 confers avirulence towards Juncea cultivars - II



Conclusions

- LemaT070880 (*AvrLmJ1*) confers avirulence towards three *B. juncea* lines tested.
 - Possibly confers species-specific avirulence
 - Corresponding R gene unknown.
- Typical characteristics of avirulence genes
 - Secretion signal, cysteine rich, no homology, located in AT-rich region and highly up-regulated *in planta*
- **RNAseq data in combination with mapping allowed identification of candidate gene**