

 Agriculture and Agri-Food Canada Agriculture et Agroalimentaire Canada



**Improving disease resistance
in Brassica: from basic science
to applied research**

Hossein Borhan
Agriculture and Agri-Food Canada, Saskatoon Research Centre

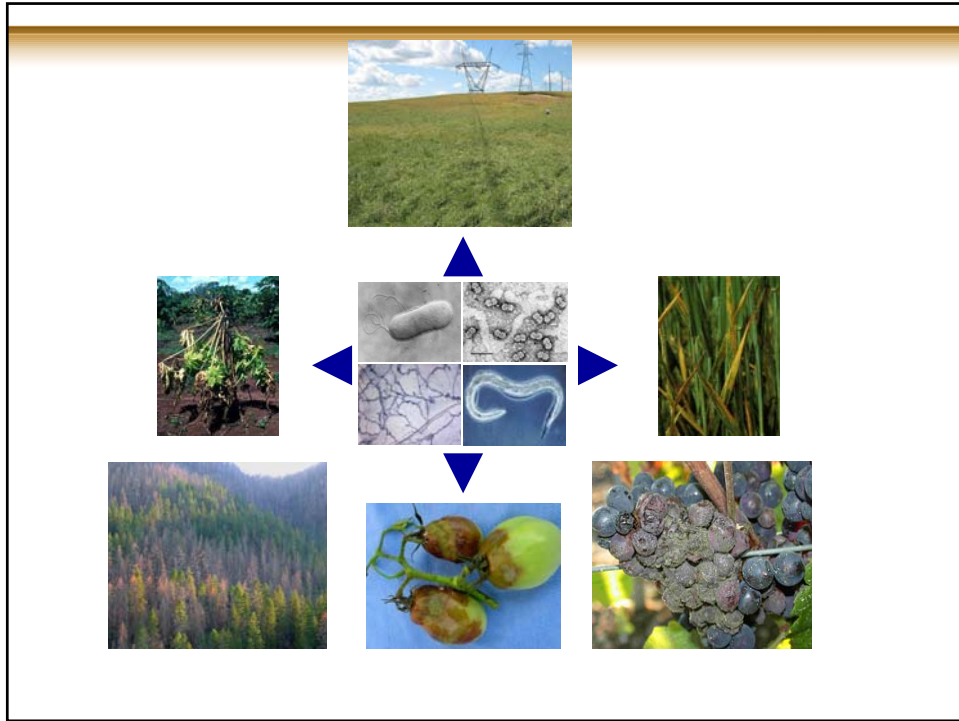
Canada

Improving disease resistance in crops

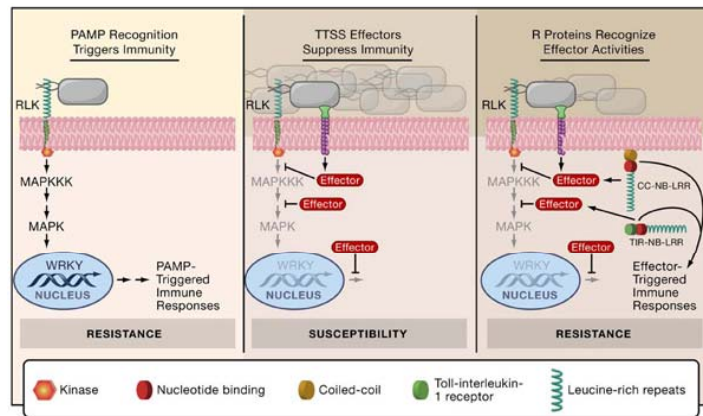
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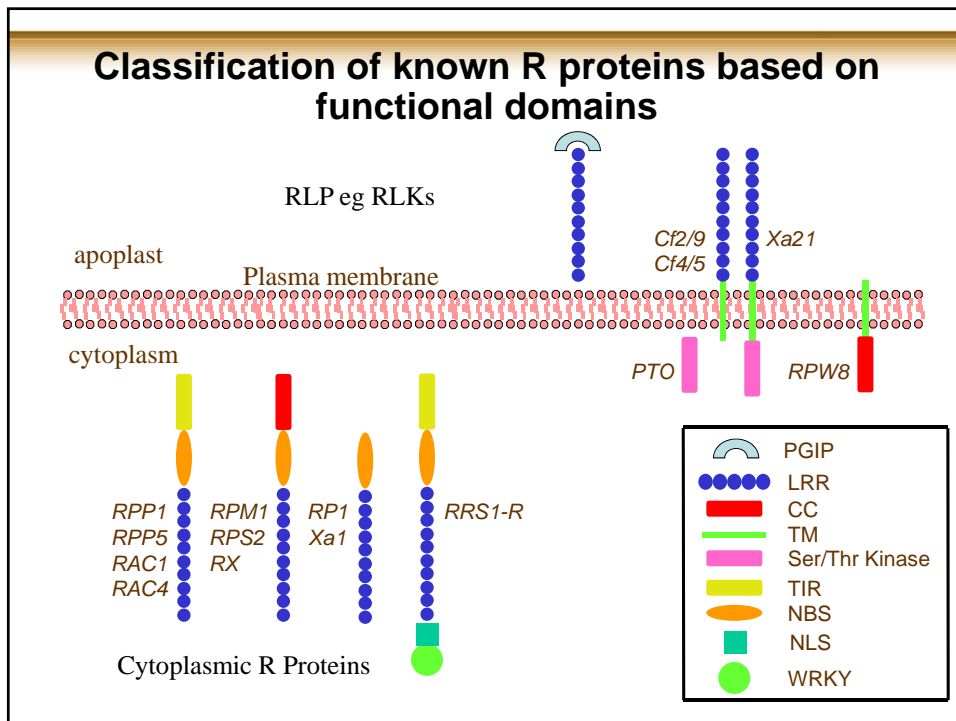
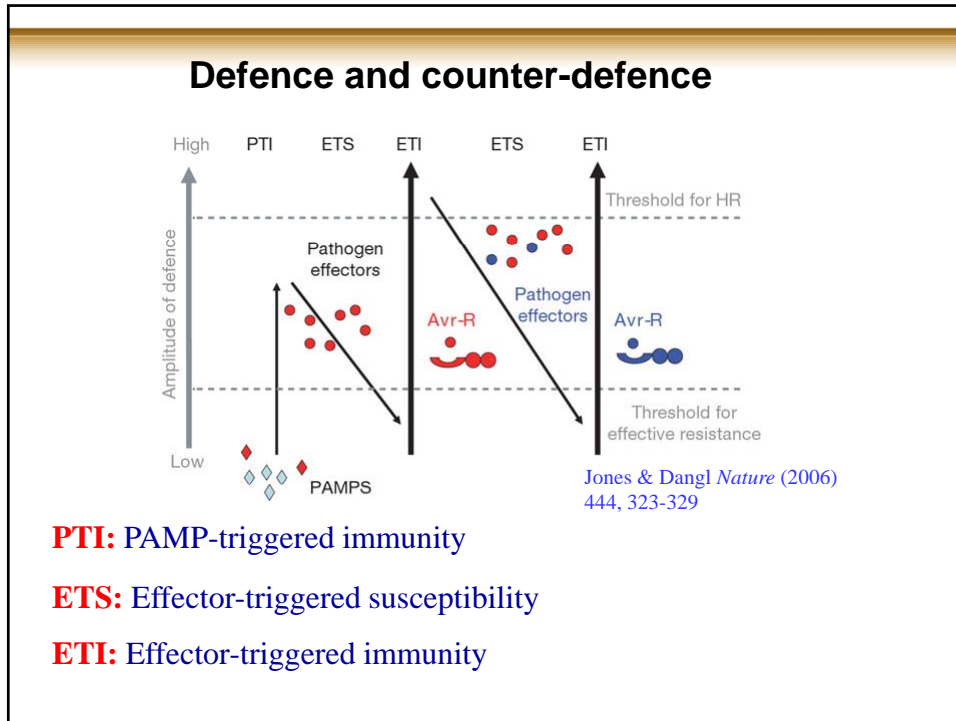
Conventional molecular pathology approaches

Deploying pathogen effectors



Plant perceive an invading pathogen by detecting Pathogen Associated Molecular Pattern (PAMP) and pathogen effectors





Improving disease resistance in crops

By:

Conventional molecular pathology approaches

**White rust resistance (WRR) in Arabidopsis
and its application to Brassica**

White rust on *Arabidopsis thaliana*



<u>A.candida isolates</u>	<u>Host species</u>
Ac2	B. juncea
Ac7	B. rapa
AcBr	B.rapa (Broccoli raab)
AcBc	B. carinata
AcCs	C. sativa
Acem1	A. thaliana
AcCap	C. bursa-pastoris

Response of Brassica to different isolates of A. candida

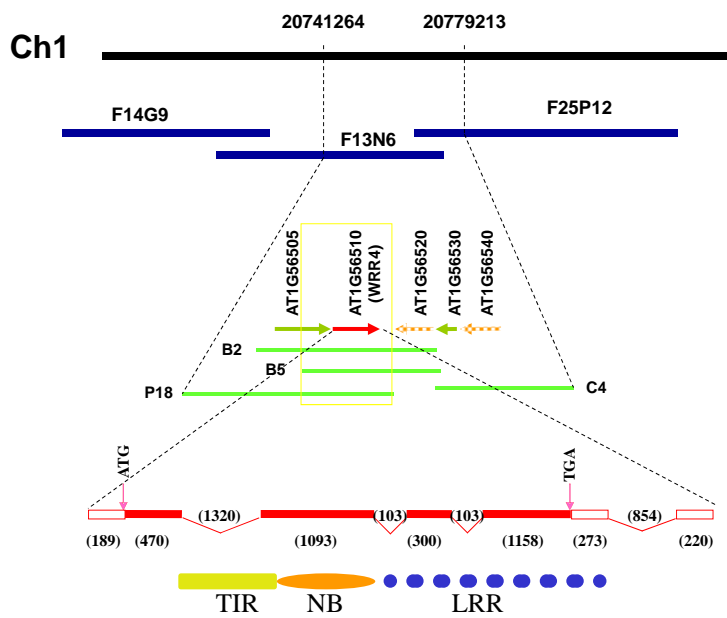
	Ac2	Ac7	AcBr	AcBc	AcCs
B. rapa	R	S	S	R	R
B. juncea	S	R	R	R	R
Broccoli	R	S	S	nt	nt
B. carinata	R	R	R	S	R
B. napus	R	R	R	R	R
C. sativa	R	R	R	R	S

Response of A.t accessions to different isolates of *A. candida*

	Ac2	Ac7	AcBr	AcCs	Acem1
Col	R	R	R	R	S
Ws	S	S	S	S	S
Ler	R	R	R	R	S
Ksk-1	R	R	R	R	R
Col-eds1 (RNAi)	S	S	S	S	S

WRR4
RAC1

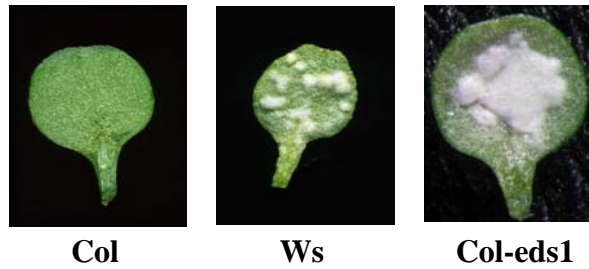
Location of WRR4 on Arabidopsis chromosome one



Arabidopsis resistance to Brassica isolates of *A. candida*

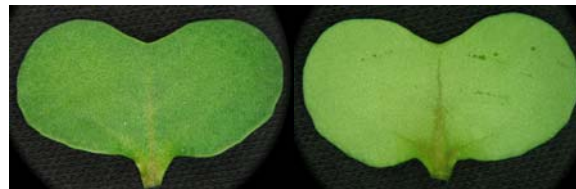
WRR4 a TIR-NB-LRR gene in *Col* confers broad spectrum resistance to several isolates of *A. candida* (Ac2, Ac7, AcBr, race9 and race4)

WRR4 resistance requires expression of the enhanced disease susceptibility 1 (*eds1*) gene

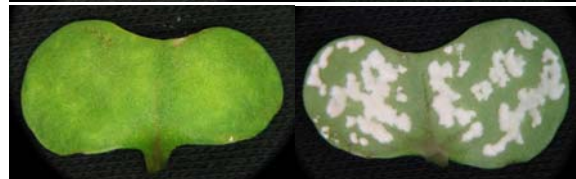


Brassica defense against Ac2 and Ac7 is *eds1* dependent

WT *B. napus*



B. Napus
Eds1-RNAi



Arabidopsis WRR4 confers resistance against Ac2

W.T B. Juncea

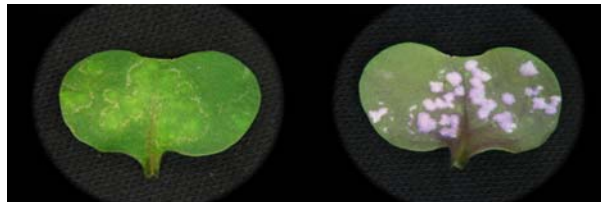


**B. juncea /A.t
WRR4**

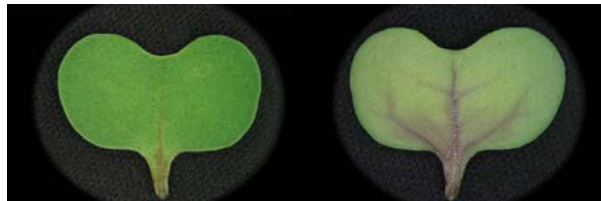


Arabidopsis WRR4 confers resistance against Ac7

W.T ACS-N32



ACS-N32/A.t WRR4



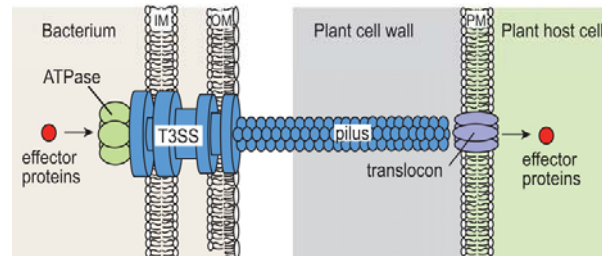
Improving disease resistance in crops

By:

Deploying pathogen effectors

**How Pathogens deliver their
effectors into the host cell?**

Bacterial Pathogens deliver effectors by T3SS



Büttner & He Plant Phys.(2009)
150,1656–1664

T3SS: Type three secretion system

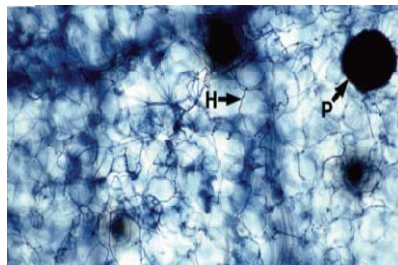
IM: Inner membrane

OM: Outer membrane

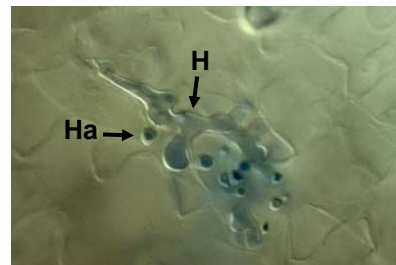
PM: Plasma membrane

Delivery of effectors by fungi and oomycete

L. maculans



A. candida

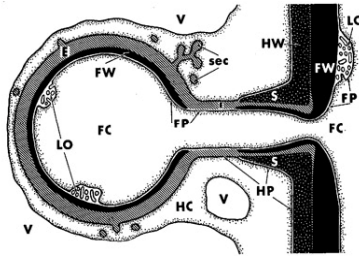
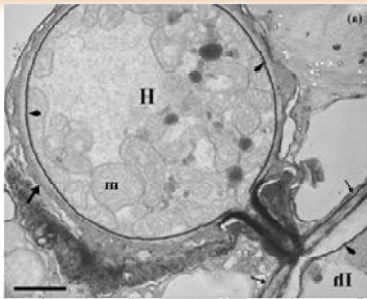


Saal et al., Plant Breeding 123, 505—511 (2004)

H: Hypha

P: Pycnidium

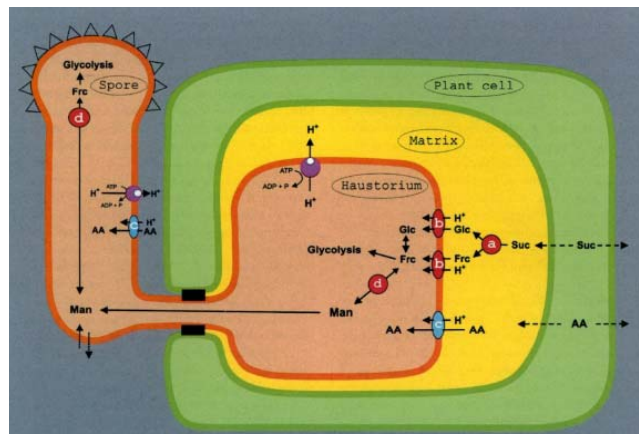
Ha: Haustorium



- FC:** Fungal cytoplasm
- FP:** Fungal plasma membrane
- FW:** Fungal cell wall
- HC:** Host cytoplasm
- HP:** Host plasma membrane
- S:** Collar like sheath
- E:** Encapsulation
- V:** Host vacuole

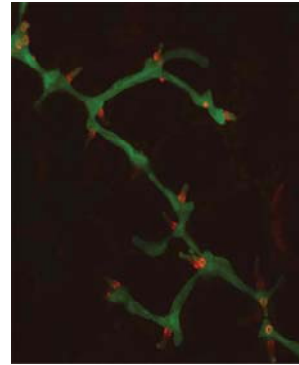
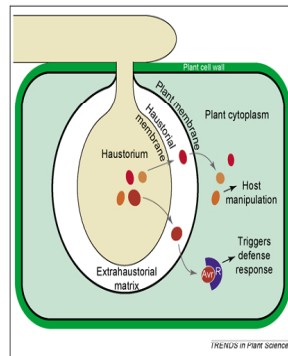
Mycopathologia 158, 457-464, 2004

Haustorium role in nutrient uptake



Voegelé and Mendgen, New Phytologist, 2003, 159:93-100

Haustoium and secretion of effectors

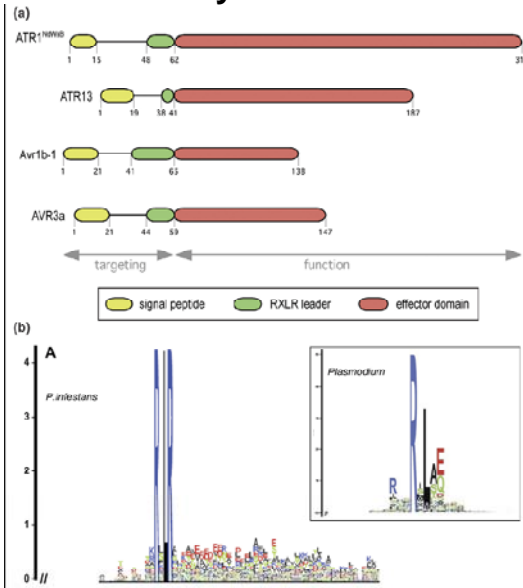


AVr3a from *Phytophthora* is localized to haustoria

Whisson et al., 2007 *Nature*, 450, 115-119

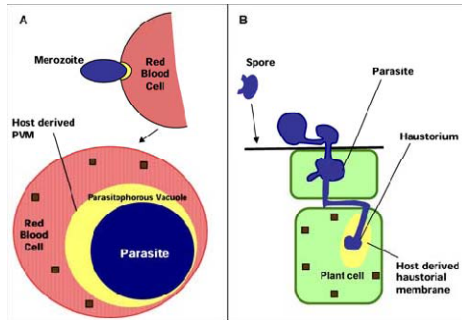
**Oomycete effectors contain a
host translocation motif**

RXLR-dEER motifs as translocation signal for delivery of oomycete effectors into plant cell

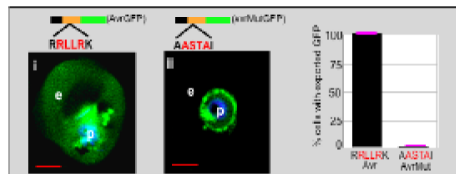


William Morgan¹ and Sophien Kamoun, Current Opinion in Microbiology (2007) 10:332–338

The oomycete RXLR and Plasmodium protein translocation motifs have been shown to be interchangeable.



RXLR motif of *P. infestans* Avr3a could be replaced by host translocation signal of Plasmodium or RXLR of *H. parasitica*



Grouffaud et al., Microbiology (2008) 154, 3743-3751

Bhattacharjee et al., (2006) PLOS pathogen

Discovery of effectors from Brassica Pathogens

Albugo candida

<u>A.candida isolates</u>	<u>Host species</u>
Ac2	B. juncea
Ac7	B. rapa
AcBr	B.rapa (Broccoli raab)
AcBc	B. carinata
AcCs	C. sativa
Acem1	A. thaliana
AcCap	C. bursa-pastoris

A. candida effectoromics

A. candida race 2v (Ac2v) has been used for

- EST sequencing
- Genome Sequencing (Next Gen. Seq.)

Transcriptome of Ac2v

A cDNA library from infected seedlings of *B. juncea* cv cutlass (susceptible to Ac2v)

Close to 35,000 cDNA sequenced both direction

*** 14510 Ac2V ESTs**

*** 35738 *B. juncea* ESTs**

A full length normalized cDNA library from spores

*** 27547 ESTs from spore**

Close to 42,000 Ac2v EST from spore and during infection

*** 24,000 Transcripts**

Genes with higher expression during infection

Transcript	Infected vs Spore	Predicted protein	Secreted	Blast hit
332_2_1	207 : 0	unknown	y	n
332_3_1	109 : 1	unknown	y	n
332_5_1	128 : 1	annexin	n	y
332_7_1	111 : 1	unknown	y	n
332_4_3	87 : 2	unkwon	n	y (Phy.)
332_9_1	88 : 1	unkown	y	n
332_6_1	87 : 4	unknown	y	y (Phy.)
332_10_1	86 : 1	unknown	y	y
332_16_1	47 : 0	unkown	y	n
332_17_1	38 : 1	unkown	y	n
332_19_1	41 : 1	CBEL	y	y (Phy.)
332_23_1	34 : 1	unknown	y	n
332_26_1	35 : 0	unkown	y	n
332_29_1	34 : 6	Sugar transporter	y	n
332_34_1	28 : 2	unkown	y	n
332_32_1	29 : 0	unkown	y	n

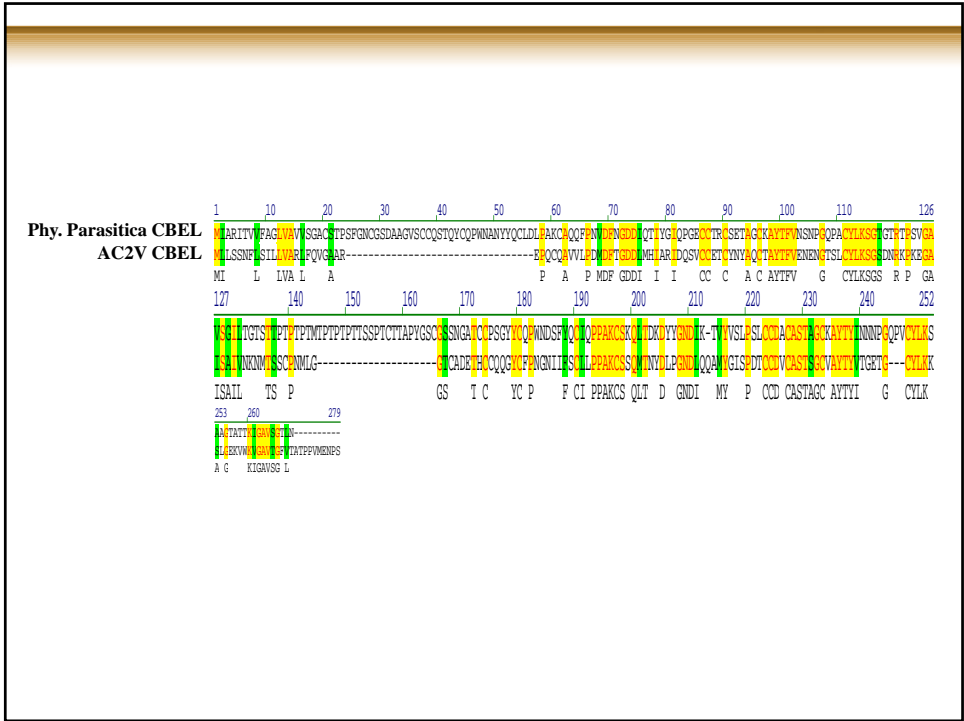
RXLR

<u>effector</u>	<u>location</u>	<u>Albugo</u>
Hydrolytic enzymes	apoplast	✓
Enzyme inhibitors	apoplast	✓
Elicitins	apoplast	✓
NLPs	apoplast	—
RXLR	cytoplasm	✓
CRINKLER	cytoplasm	?

A. candida an obligate biotroph as revealed by EST

Oomycete PAMPs and elicitors reported to induce cell death are absent, non-functional or less frequent in *A. candida*

	<u>Phy.</u>	<u>Albugo</u>
NEP	50-60	No EST
Elicitin	17	2 cDNA
Crinckler	>100	1 EST
CBEL	✓	✓
transglutaminase	✓	✓

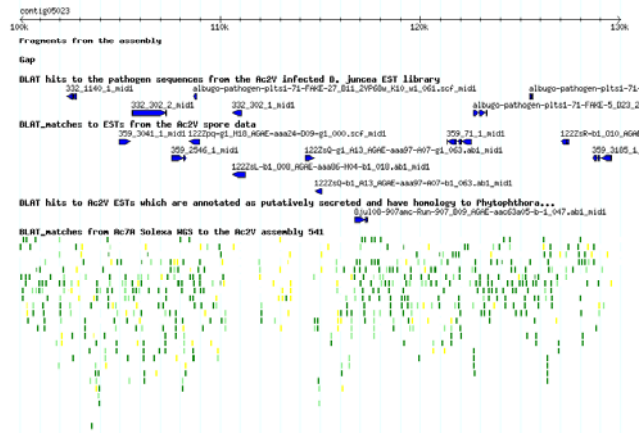


Ac2V Genome Sequencing

2 x GS-FLX + 1.5 x titanium

# of reads	2.15 million
# Mbps	694
# Mbps assembled	36.7
# contigs	4807
Avg contig length (bps)	7627
N50 length (Kb)	51
Largest contig (Kb)	316
Estimated size (Mbps)	34
Estimated coverage (fold)	14.8

Gbrowse view of a contig from *A. candida* race 2v



Potential effectors from Ac2V

Secreted proteins

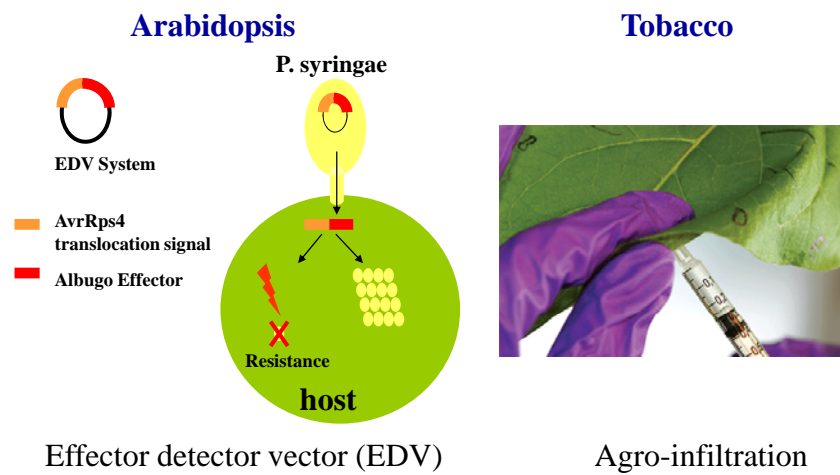
* 802 genes coding for proteins
larger than 100 aa with SP

* 204 secreted proteins with RXLX domain

Functional assay of effectors by transient expression

Example from *A. candida*

High throughput screening of Ac effectors using transient assay in *Arabidopsis* and tobacco



Functional assay of effectors by transient expression

Example from *Scelrotinia sclerotiorum*

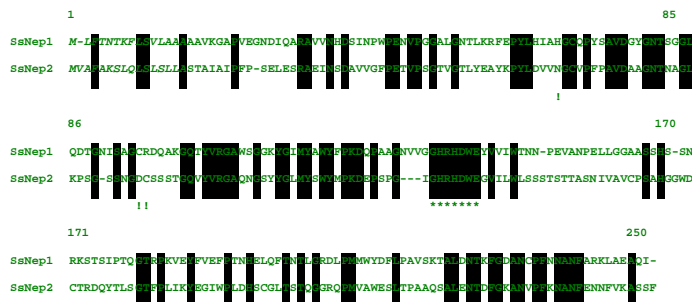
Identifying Necrosis and ethylene inducing protein (Nep) from *Sclerotinia sclerotiorum*

**Nep a 24 kDa protein was first identified from
*Fusarium oxysporum***

Nep triggers cell death in plant

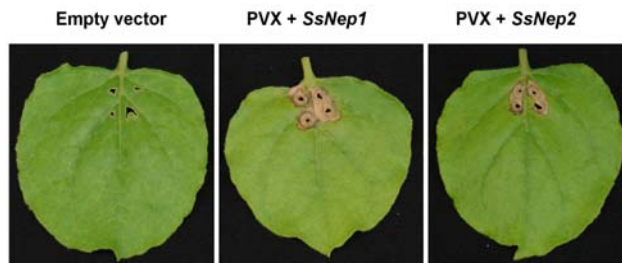
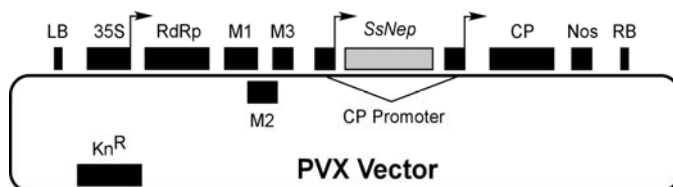
Nep is present in fungi, oomycete and bacteria

S. sclerotiorum has two Nep genes



Dallal Bashi et al., 2009
 Mol. Plant Path.

Induction of cell death by SsNep in tobacco



Dallal Bashi et al., 2009
 Mol. Plant Path.

Leptosphaeria maculans effectomics

Leptosphaeria maculans denovo sequencing

1x 454 titanium for LM isolates WA74 and WA51

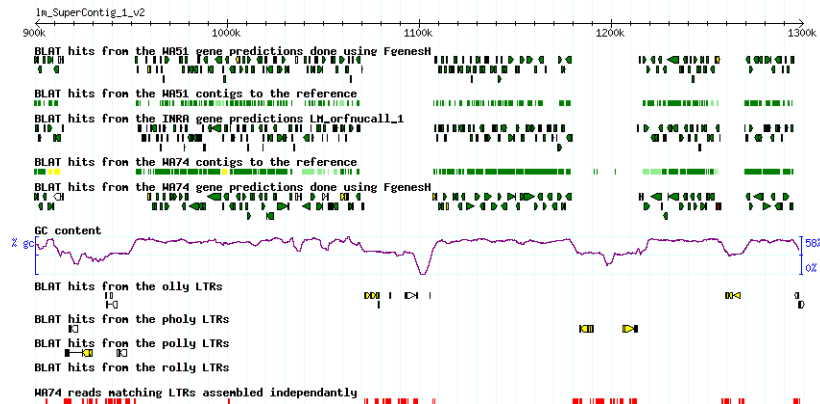
	<u>WA74</u>	<u>WA51</u>
contigs	8315	11908
# Mbps	45.3	29.2
Mean length (bp)	5448	2452
N50 (bp)	11397	3901
Largest (bp)	96907	37152
Q40	98.2%	95.1%
# all contigs	9329	22969
# Mbps in all	45.6	32.2

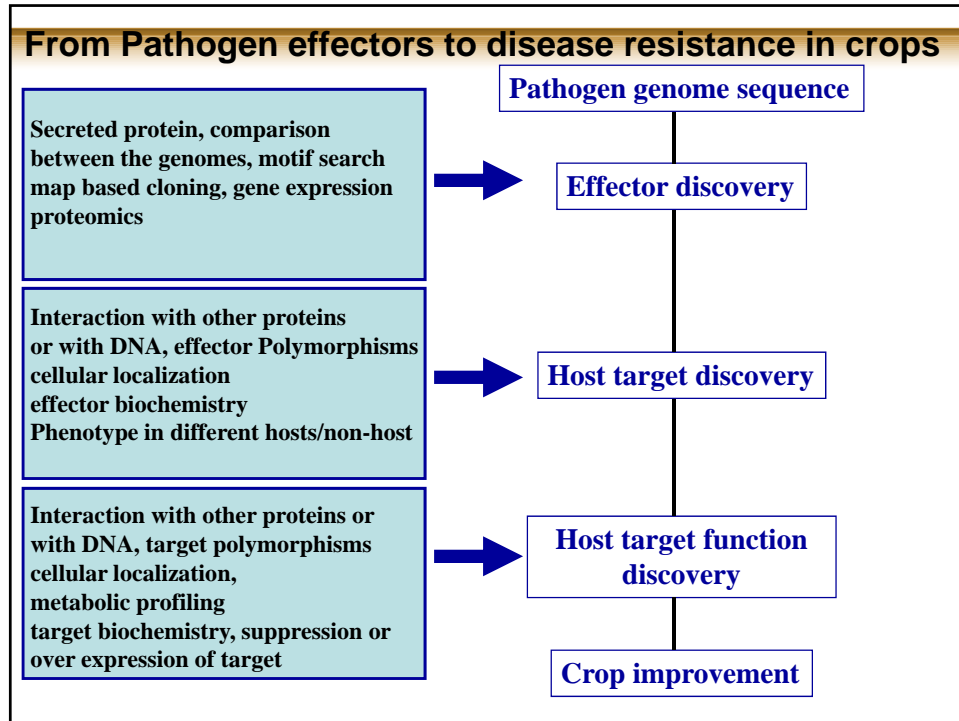
LM isolate JN3 has been sequenced and assembled through LM genome project at INRA

WA74 and WA51 alignment with JN3

Tools and resources developed for Ac2V effectors could be applied to identifying LM effectors

Gbrowse view of a contig from *L. maculans*





Summary

Information from resistance and defense in Arabidopsis could be directly used to improve resistance to pathogens in crops.

Transient expression of pathogen effectors in plant, an efficient and high throughput approach to identify novel sources of resistance.

Future works:

Sequencing additional isolates and different species of A.C and L.M

Sequencing P. brassica pathogen of clubroot

Comparative pathogen genomics

Lab members

**Jan Utermark
Elena Beynon
Colin Kindrachuk
Derek Howse**

Bioinformatic group (SRC)

**Matthew Links
Steven Karcz
Wayne Clarke
John Nixon**

Collaborators:

Andrew Sharp (PBI)
Dwayne Hegedus (SRC)
Derek Lydiate (SRC)
Eric Holub (Warwick, HRI)
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