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## **Avirulence proteins of *Leptosphaeria maculans* involved in suppressive interactions share a common structural pattern and are part of a larger family**

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# Avirulence proteins of *Leptosphaeria maculans*, involved in suppressive interactions, share a common structural pattern and are part of a larger family

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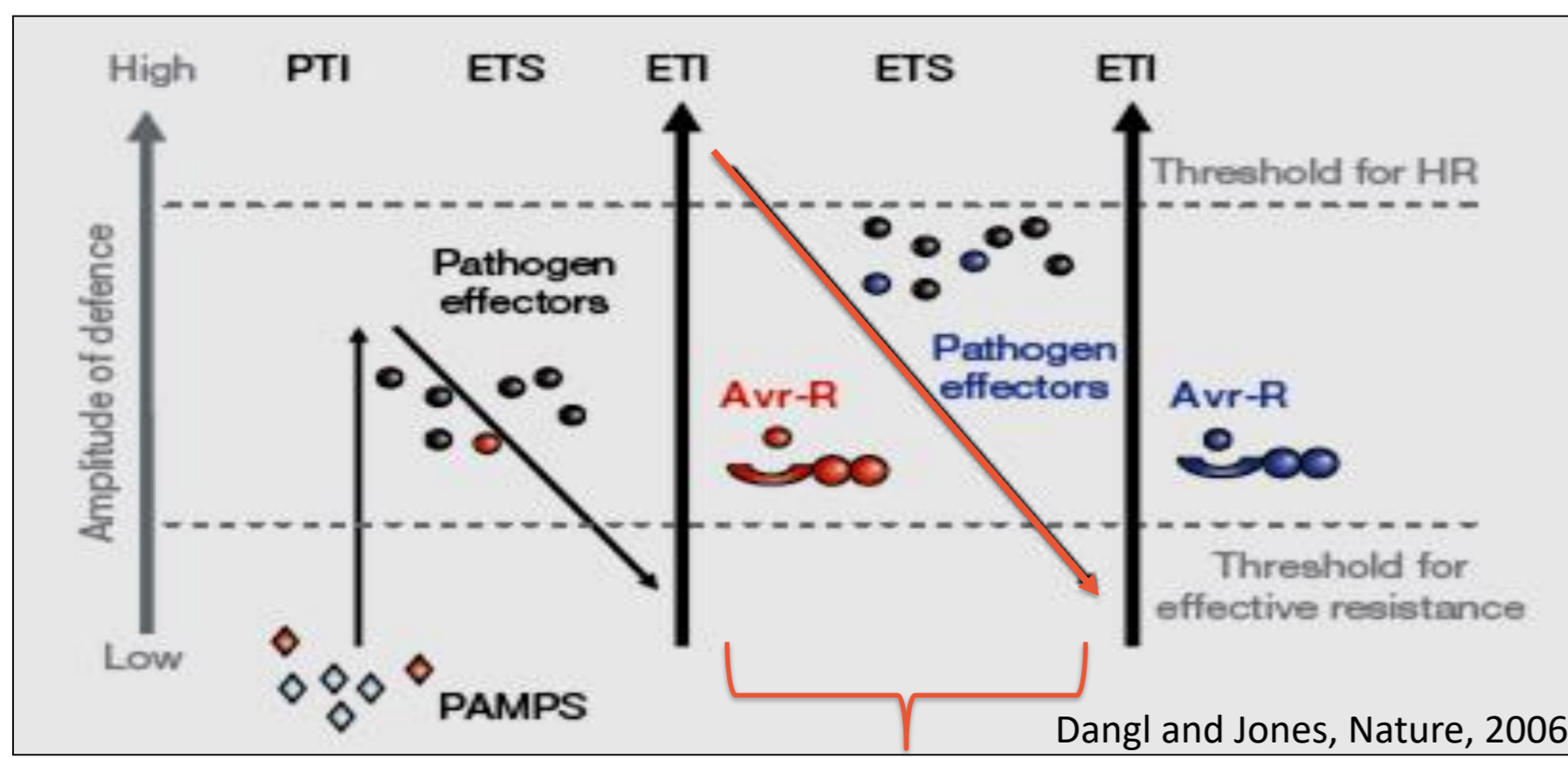
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ECFG15  
ROME • ITALY 2020

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## How pathogens escape Effector-Triggered Immunity



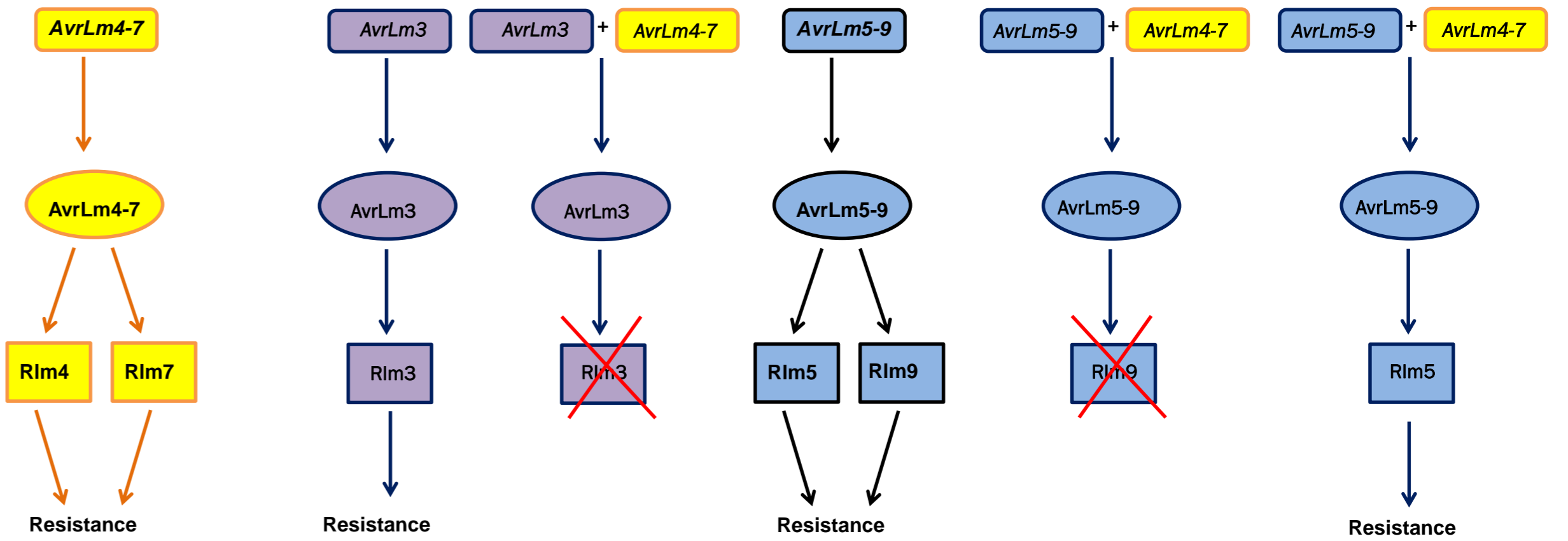
- Complete deletion of the avirulence (AVR) gene
- Down-regulation of the AVR gene
- Point mutations in the AVR gene
- Acquisition of new effectors able to suppress recognition

Two examples of AVR proteins suppressing Effector-Triggered Immunity in fungi:

- AVR1 in *Fusarium oxysporum* suppressing recognition of AVR2 by I2 and of AVR3 by I3 (Houterman et al., PLOS Pathog. 2008)
- AvrLm4-7 in *Leptosphaeria maculans* (Plissonneau et al., PLOS Pathog. 2016; Ghanbarnia et al., Mol. Plant Pathol. 2018)

## Suppressive interactions between avirulence genes in *L. maculans*

- The Dothideomycete *Leptosphaeria maculans* is responsible for stem canker of oilseed rape.
- The AVR gene of *L. maculans* AvrLm4-7 suppresses the recognition of AvrLm3 and AvrLm5-9 by the resistance proteins Rlm3 and Rlm9, respectively.
- AvrLm3 and AvrLm5-9 are always present in natural isolates of *L. maculans*.
- The presence of AvrLm4-7 does not impede AvrLm3 and AvrLm5-9 expression.
- The three AVR do not physically interact.



Goal: determine the mechanism involved in ability of AvrLm4-7 to suppress ETI using structural biology

## Production of *L. maculans* effectors

Crystal structure of AvrLm4-7 had been previously determined (Blondeau et al., Plant J. 2015). AvrLm5-9 recombinant protein was expressed in *Pichia pastoris* (pPIC2a vector) and its crystal structure determined. AvrLm3 could not be produced either in *P. pastoris* or *E. coli*.

Resolution of AvrLm5-9 structure



Low production of AvrLm3

## Ecp11-1, an homologue of AvrLm3, triggers recognition by a resistance gene of oilseed rape

Homologues of AvrLm3 identified in other fungal pathogens including Ecp11-1, apoplastic effector of *Cladosporium fulvum* (*Passalora fulva*) triggering cell death on wild tomato.

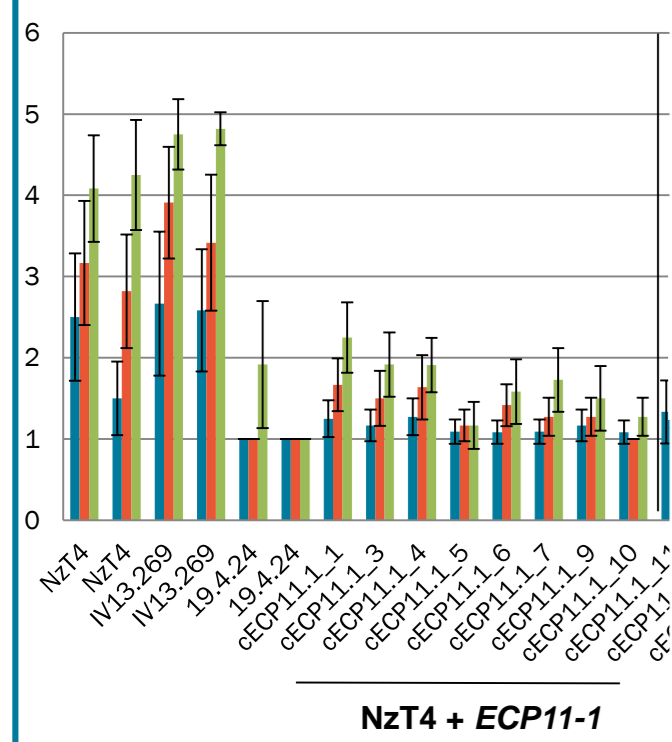
Sequence homology between AvrLm3 and Ecp11-1 (35 % identity)

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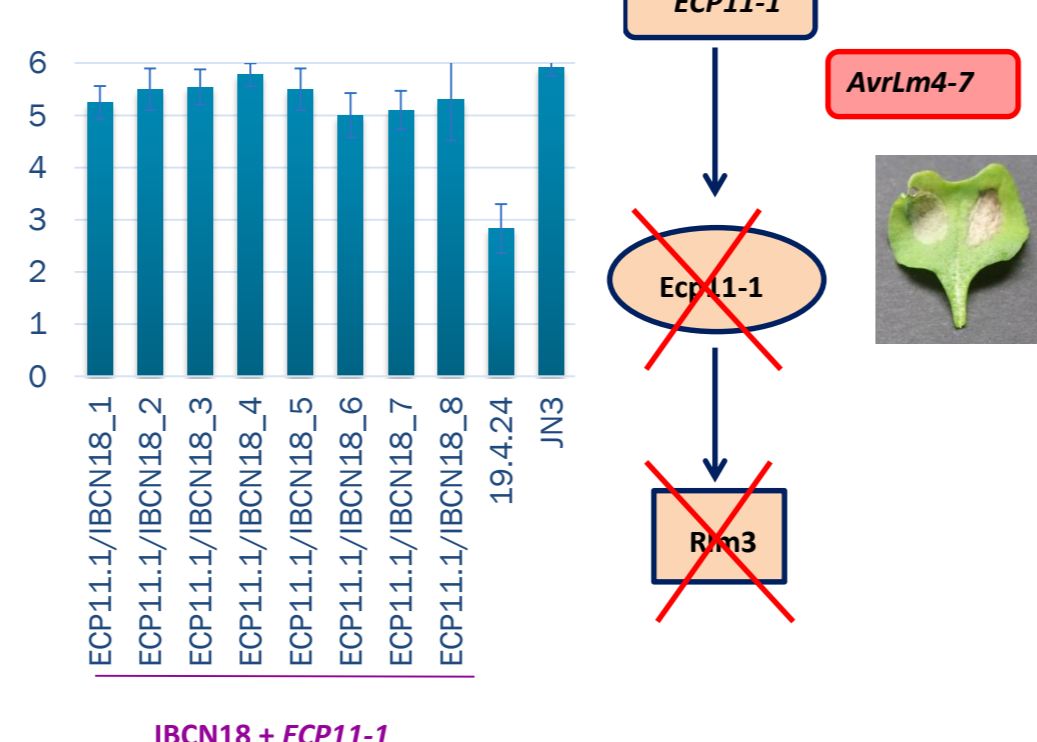
AvrLm3      MKPTVIQIIFLFTAFFARTL--E--HVAFAFSSDQSLGRNE--NLK--SKNMLSI
Ecp11-1     MLSSAKTLMLL--SML--AYTKPKAYSLEKVAALKWVQRIIPGGDNCNFKSYDSLYQQ
**          *:::          *:::          *:::          *:::          *:::
AvrLm3      ---SRVLLDDEFGDDGFSLLTITD--IS--E--TG--CRCSVHTATAVRH--SGKRYE
Ecp11-1     ---LN--LWKKND--AGDAGDSTAPKIQEAP--SD--PGSKT--I--GV--CATAVRW--RNG--KWF
**          *:::          *:::          *:::          *:::          *:::
AvrLm3      RRIGVNSNLEDVAVITGRPFIPVNG--LHE---F--SRGFVSRKDEVC--DHE
Ecp11-1     DGQQ--FD--DKP--IT--EVLGRRWYDE--EADKDIYVGRGFISNDNV--CG--S
**          *:::          *:::          *:::          *:::          *:::
    
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ECP11-1 as AvrLm3 is able to trigger recognition by Rlm3 and that recognition is masked by the presence of AvrLm4-7

Mean disease rating on Rlm3 cultivar



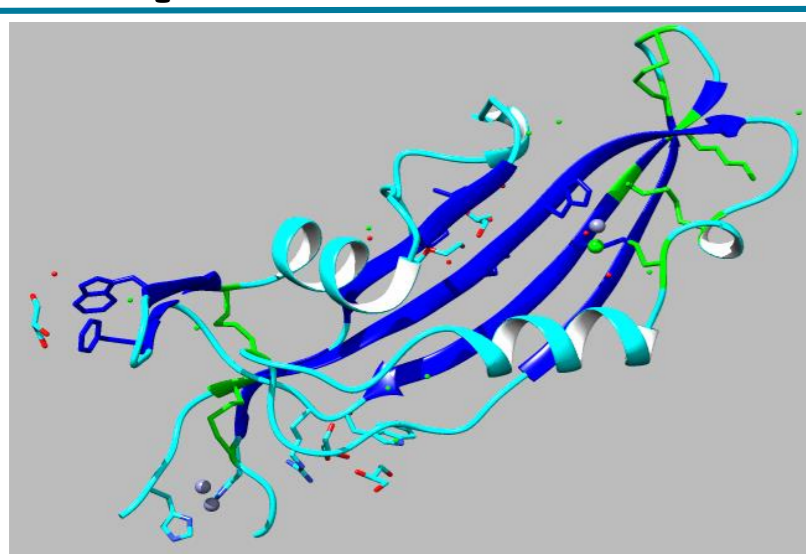
Mean disease rating on Rlm3 cultivar



- Nzt4 and V13.269 are virulent towards Rlm3
- 19.4.24 is avirulent towards Rlm3

- IBCN18 are virulent towards Rlm3 and avirulent towards Rlm7
- 19.4.24 is virulent towards Rlm3

## Resolution of Ecp11-1 structure and prediction of AvrLm3 structure



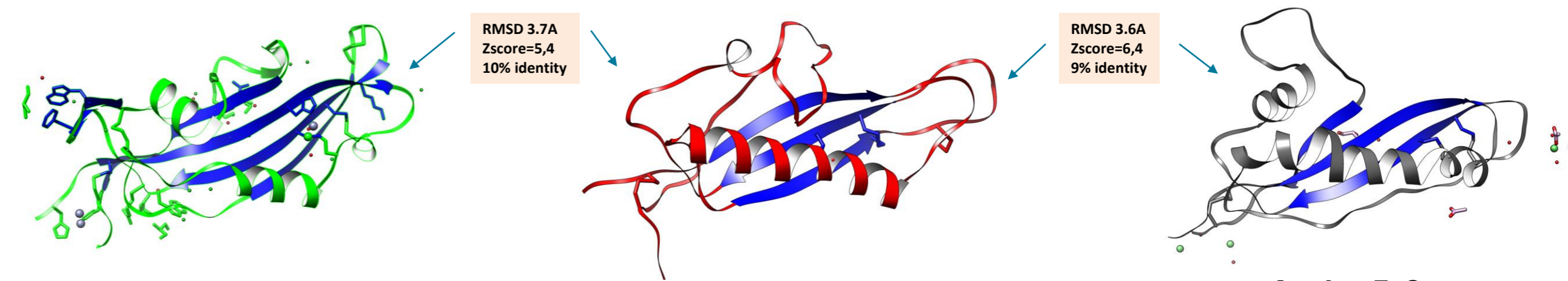
Ecp11-1 crystal structure



AvrLm3 structural model based on Ecp11-1 structure

## Identification of a common structural pattern between effectors involved in suppressive interactions

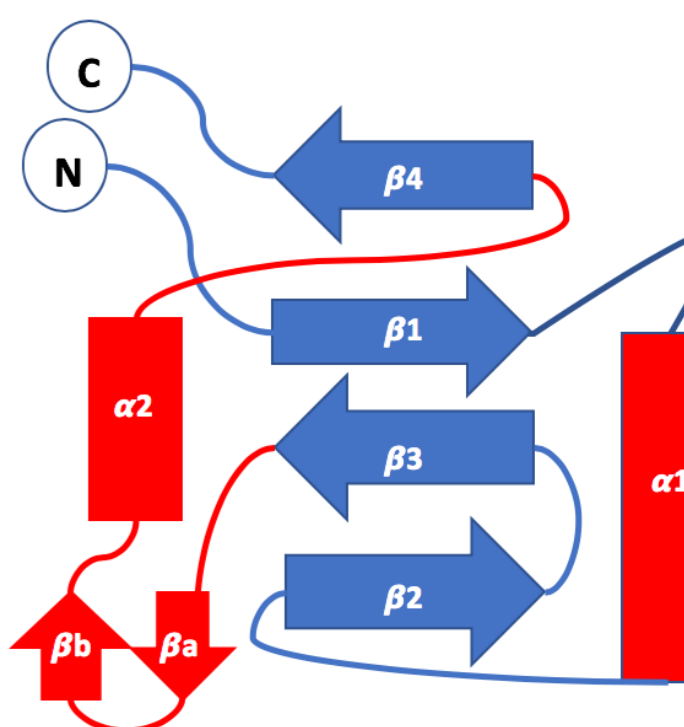
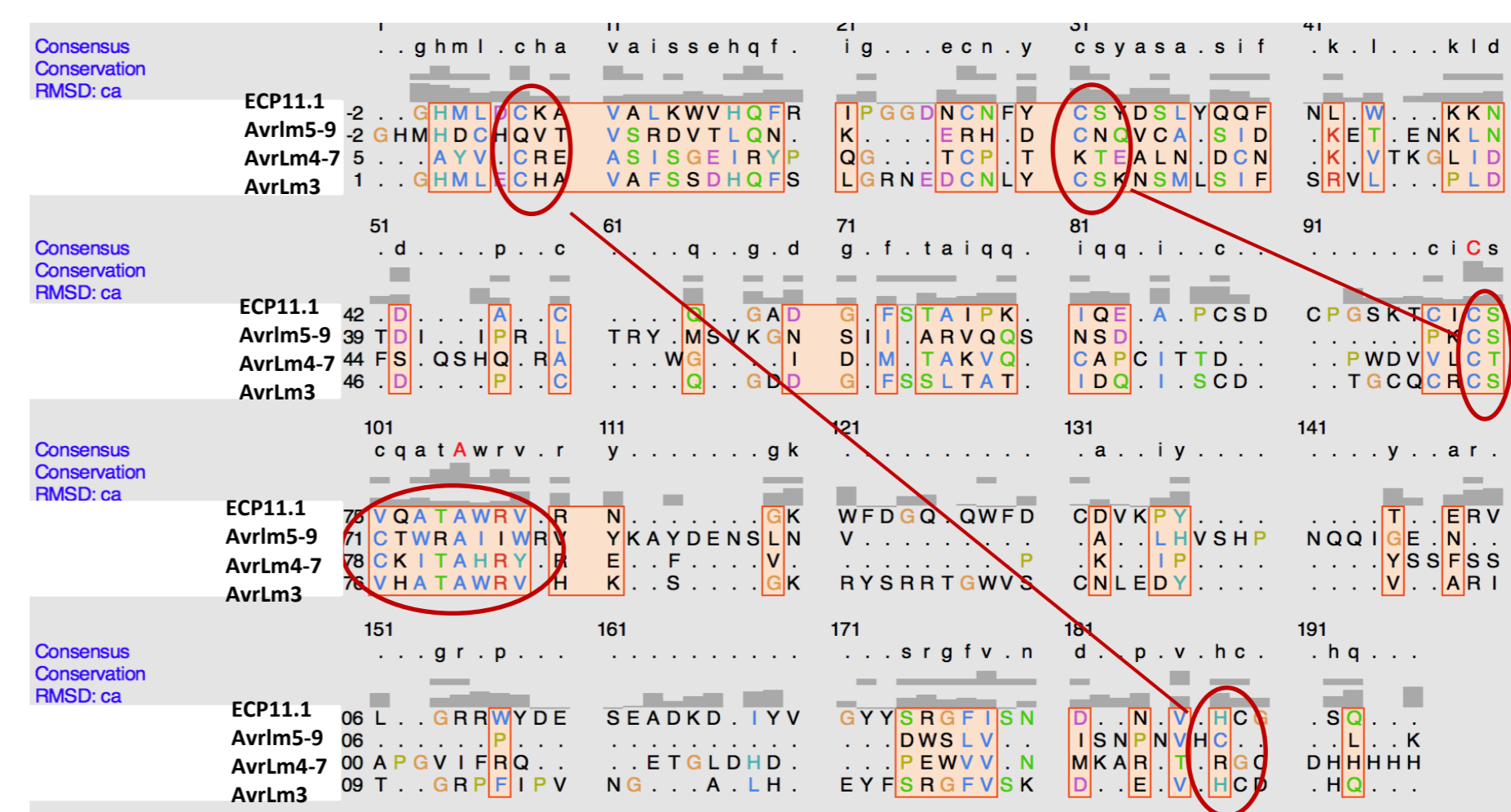
AvrLm4-7, AvrLm5-9 and Ecp11-1 (AvrLm3) share structural analogies



Ecp11-1

AvrLm4-7

AvrLm5-9

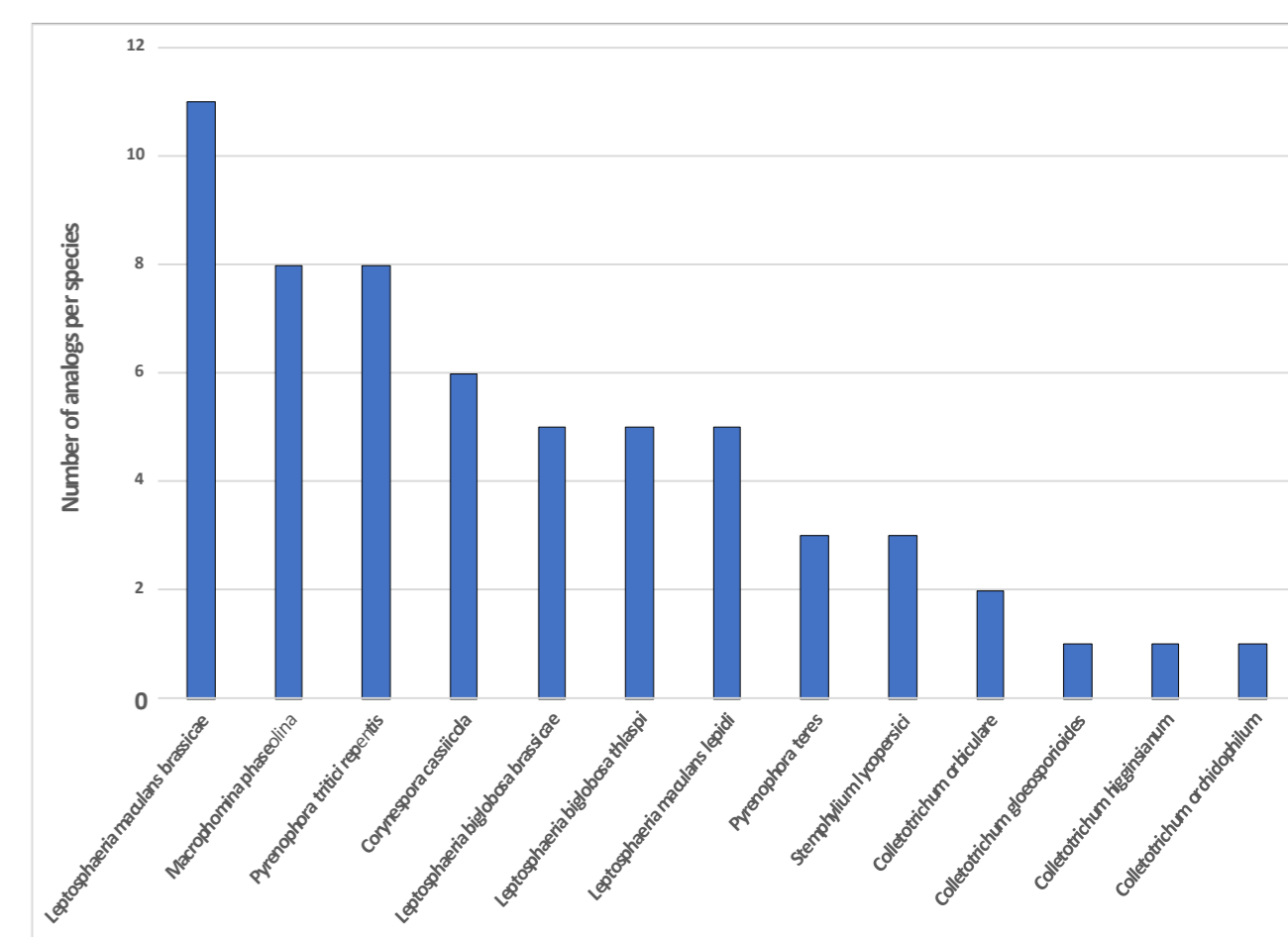


- Central  $\beta$ -sheet with the same topology
- Helical connection between  $\beta$ 1 and  $\beta$ 2 conserved
- 2 of the disulphide bonds conserved
- Connections between  $\beta$ 3 and  $\beta$ 4 very different
- 4th strand irregular in AvrLm4-7 ( $\beta$ 4) and AvrLm5-9 ( $\beta$ 2)

## Identification of a larger structural family in *L. maculans* and other phytopathogenic fungi

Structure-informed searches identified 13 putative structural analogues among *L. maculans* effector candidates (most of them located in TE-rich regions, over-expressed during primary infection). Putative structural analogues were also identified in other phytopathogenic fungi.

Accession number	Length (aa)	AVR gene	Genome compartment	Over-expression in planta	SuperContig
Lmb_jn3_03262	143	AvrLm4-7	TE-rich	Yes	3
Lmb_jn3_10106	141	AvrLm5-9	TE-rich	Yes	12
Lmb_jn3_03263	142	Gene-rich	Gene-rich	Yes	3
Lmb_jn3_01427	137	Gene-rich	Gene-rich	Yes	1
Lmb_jn3_12986	143	TE-rich	TE-rich	Yes	18
Lmb_jn3_08419	174	TE-rich	TE-rich	No	10
Lmb_jn3_00001	160	AvrLm3	TE-rich	Yes	NA
Lmb_jn3_08343	141	TE-rich	TE-rich	Yes	9
Lmb_jn3_03815	140	TE-rich	TE-rich	Yes	3
Lmb_jn3_01428	130	Gene-rich	Gene-rich	Yes	1
Lmb_jn3_01426	139	Gene-rich	Gene-rich	Yes	1
Lmb_jn3_08421	127	TE-rich	TE-rich	No	10
Lmb_jn3_08418	138	TE-rich	TE-rich	Yes	10



Conclusions : Structural family of effectors: (i) Common origin? (ii) Same plant proteins or cellular processes targeted? (iii) recognition by the same resistance proteins?

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