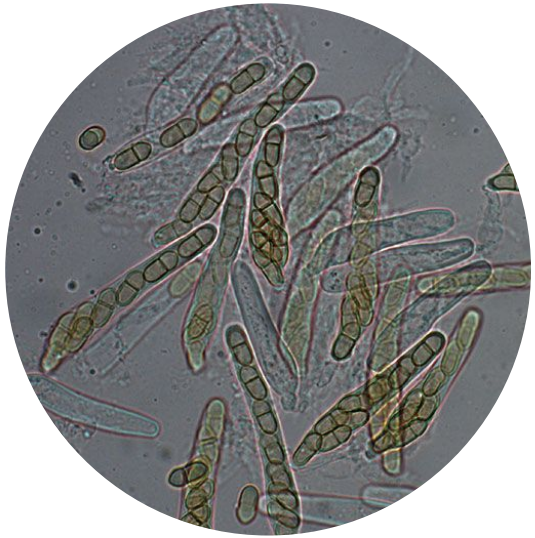
The MAX effector AvrRvi6 from *Venturia inaequalis* is recognised by the Rvi6 resistance protein in apple trees

Sirine Benmamar

Team : EcoFun

16.01.2024

Venturia inaequalis, a fungal pathogen causing apple scab



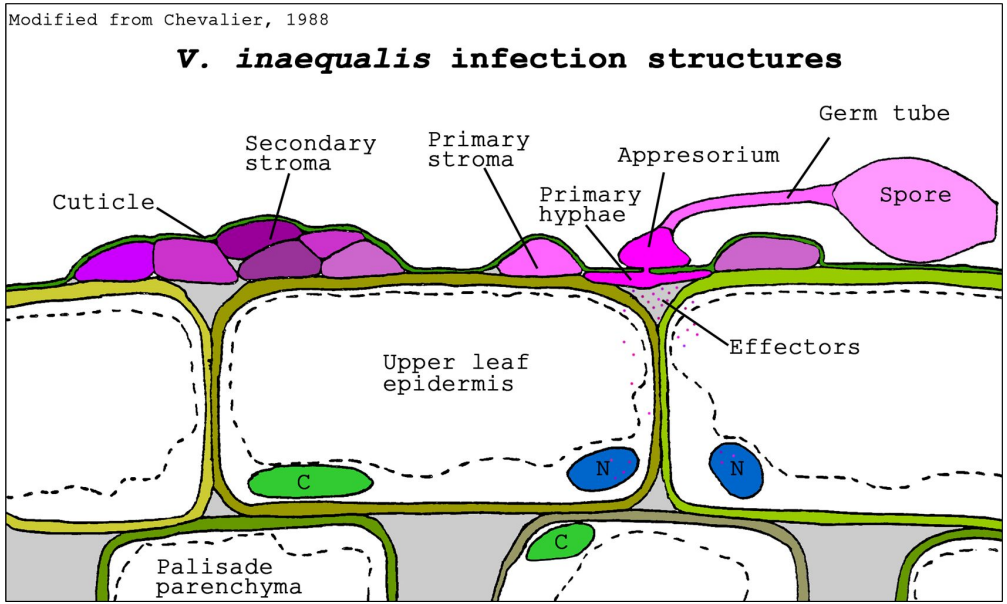
(Picture by M.-N. Bellanger, INRAe)

(Bowen *et al.*, 2010)

Apple scab is the most economically important disease of apple worldwide.

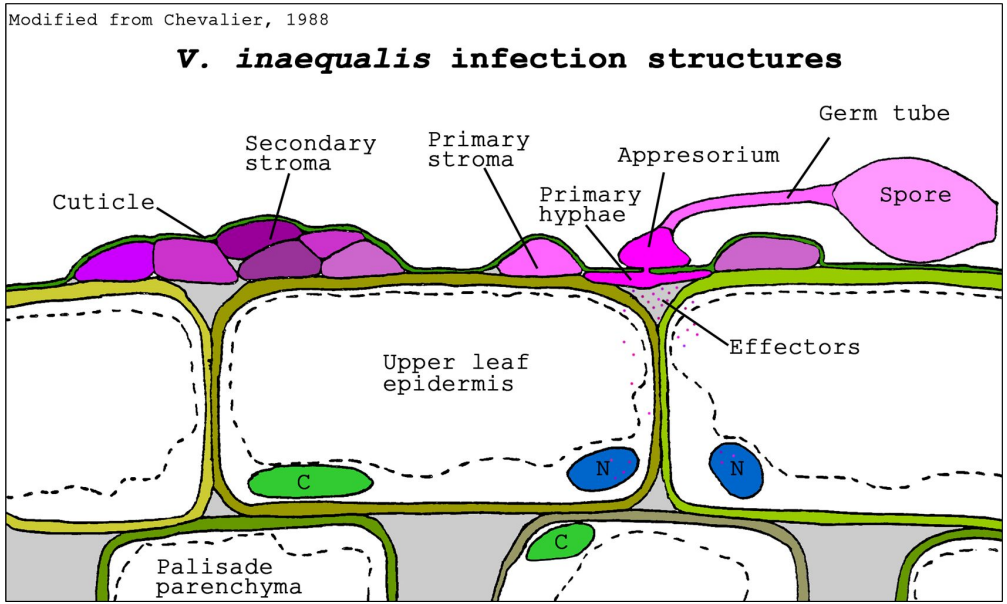
It is predominantly controlled by a combination of sanitation and cultivation measures, and heavy fungicide application.

V. inaequalis colonizes the subcuticular space of apple trees



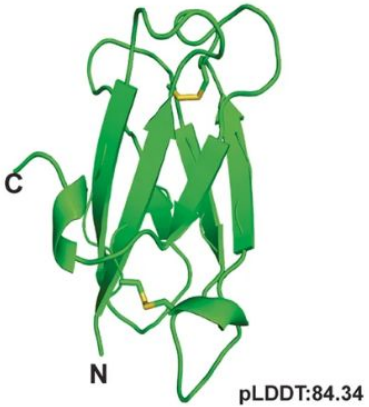
➡ **Fungal biomass accumulates in the subcuticular space prior to sporulation.**

V. inaequalis colonizes the subcuticular space of apple trees



- Fungal biomass accumulates in the subcuticular space prior to sporulation.

- *V. inaequalis* secretes at least 759 non-enzymatic proteinaceous effector candidates, 75 of which belong to the MAX-like structural family



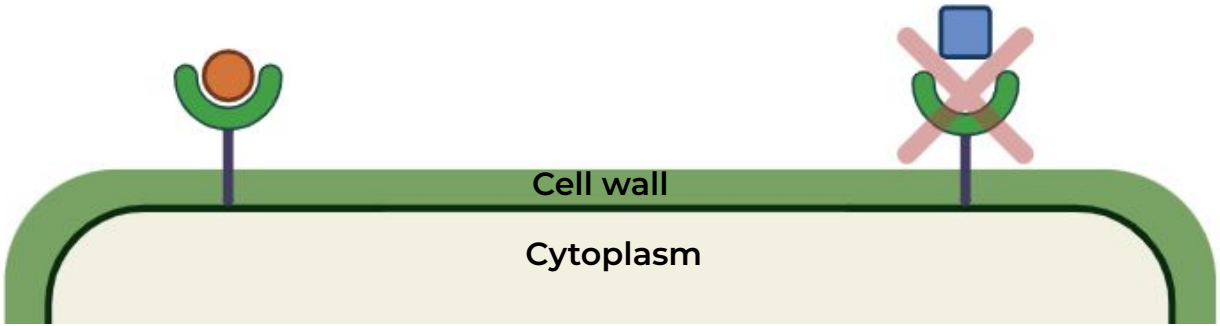
A MAX-like family representative

(Rocafort *et al.*, 2022)

A gene-for-gene relationship between *V. inaequalis* and apple

Resistance ← Recognised effector

Unrecognised effector → Susceptibility



- AvrRvi6 is a MAX effector
- Rvi6 is an RLP (with a protein sequence similar to RXEG1

RXEG1's resolved structure

(Sun *et al.*, 2022)

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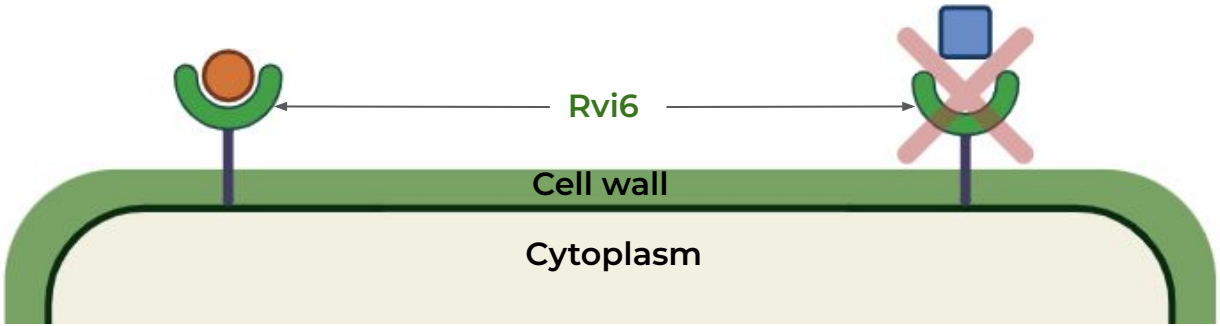

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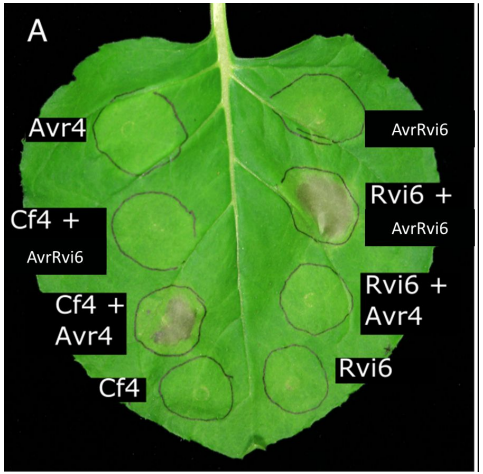
Rvi6 recognition of AvrRvi6 can be replicated in *N. benthamiana*



Cf4 and Avr4 are positive HR controls.

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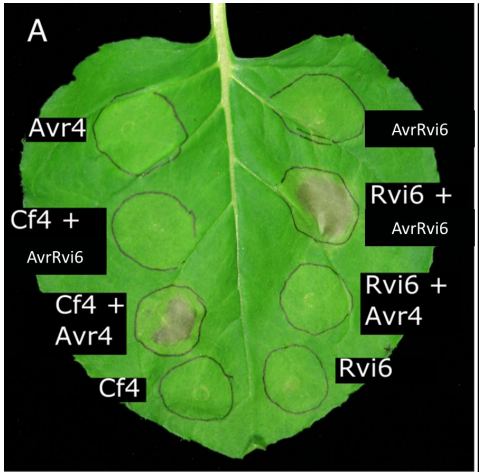

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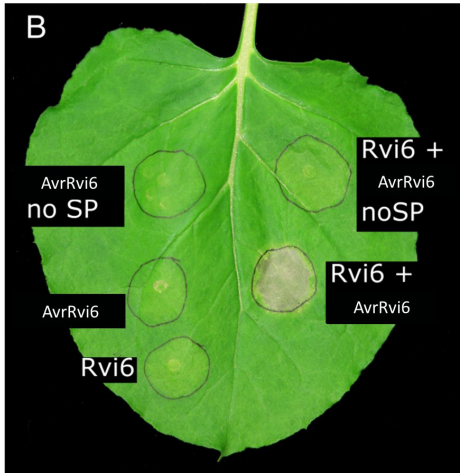
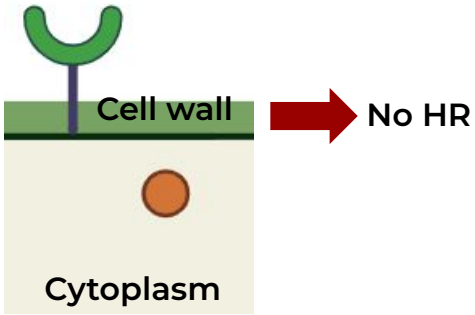
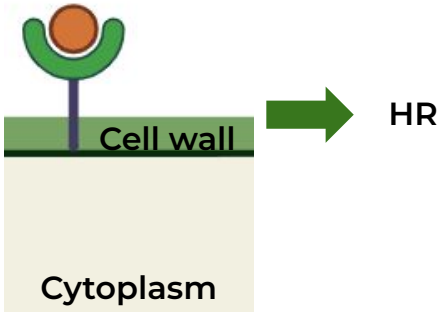


AvrRvi6 HR is dependent on the secretion of the effector in the apoplast.
(SP = secretion Signal Peptide)





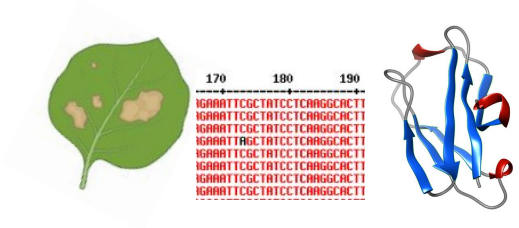
PhD project

Functional characterization of the effector AvrRvi6 from the fungal pathogen *Venturia inaequalis*

Goal Using the AvrRvi6 and Rvi6 systems as a gateway to study the MAX effectors of *V. inaequalis* in order to fill the knowledge gap on the effector biology of this pathogen.

- Study of AvrRvi6 recognition by Rvi6 (type, location, factors involved)
- Study of AvrRvi6 (impact of natural polymorphism on structure + function, virulence functions, impact on Rvi6 location)

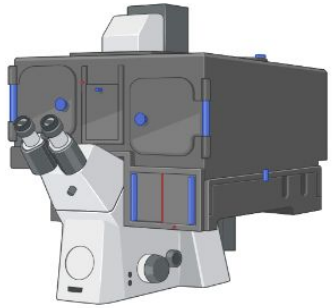
PhD project



- Heterologous expression (*N. benthamiana*)
- Polymorphism analysis
- NMR or 3D structure prediction
- Protein-protein interaction

Structure-function analysis of AvrRvi6 and Rvi6 proteins

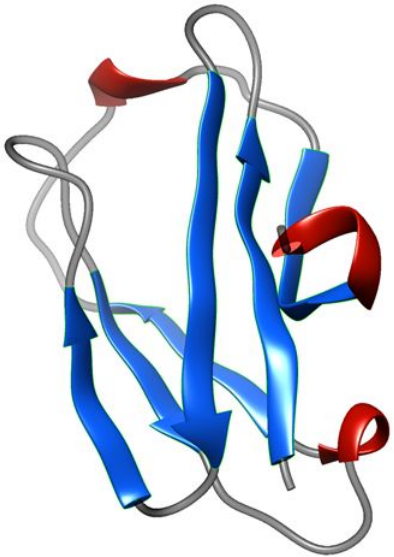
Studying the localization of AvrRvi6 and Rvi6 proteins



Complementation essay
Live imaging

AvrRvi6 is a MAX effector

The 3D structure of AvrRvi6 was determined using AlphaFold2



The structure appears to be similar to the MAX effectors family in *M. oryzae*

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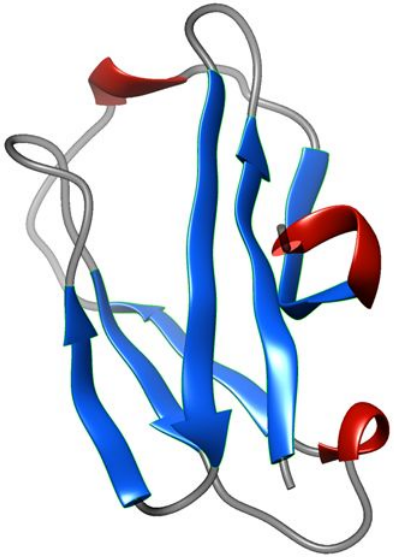


This was verified through a structural homologs search (using DALI)

6R5J

New MAX Effector from Magnaporthe oryzae

PDB DOI: https://doi.org/10.2210/pdb6R5J/pdb

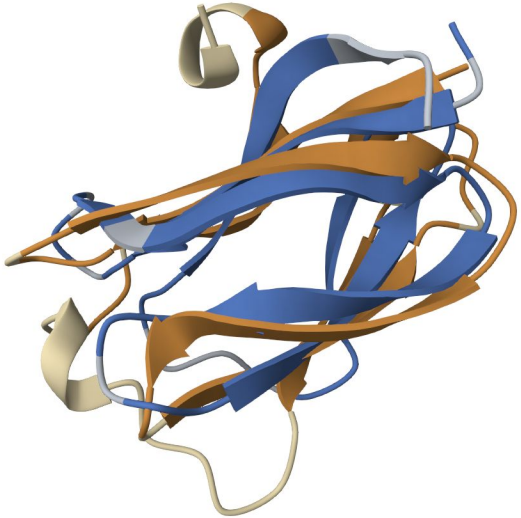
Classification: IMMUNOSUPPRESSANT
Organism(s): Pyricularia oryzae P131
Expression System: Escherichia coli
Mutation(s): No

Deposited: 2019-03-25 **Released:** 2020-05-06
Deposition Author(s): Hoh, F., Padilla, A., De Guillen, K.
Funding Organization(s): French National Research Agency

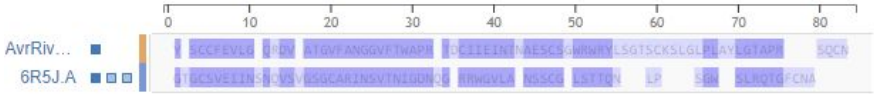
- The structure appears to be similar to the MAX effectors family in *M. oryzae*
- The structurally closest protein to AvrRvi6 is a MAX effector, 6R5J, from *M. oryzae*

AvrRvi6, a MAX effector

Structural alignment of 6R5J (a MAX, in blue) and AvrRvi6_B04



```
AvrRvi6_B04  --YSCCFEVLG-QRDV-ATGVFANGGVFTWAPRTDCIIEINTNAESCSGWRWRYLSGTSCKSLGLPLAYLGTAPR---SQCN
6R5J         qgTGCSVEIINsNQVSvGSGCARINSVTNIGDNQGRRWGVLA-NSSC-GLSTTQ-------nlPSGW-SLRQTGFcna----
                      :         :         :         :         |         :         :         :
AvrRvi6_B04  --LEEEEEEL-LLLE-EEEEEELLLEEEEEELLEEEEELLLLLHHHLEEEELLLLLLHHHLLLLLEEEEELH---HHHL
6R5J         llLEEEEEEELlLLLEeEEEEEELLEEEEELLLLLEEEEEE-LLLL-LEEELL-------llLLLL-EEEEEEEell----
```

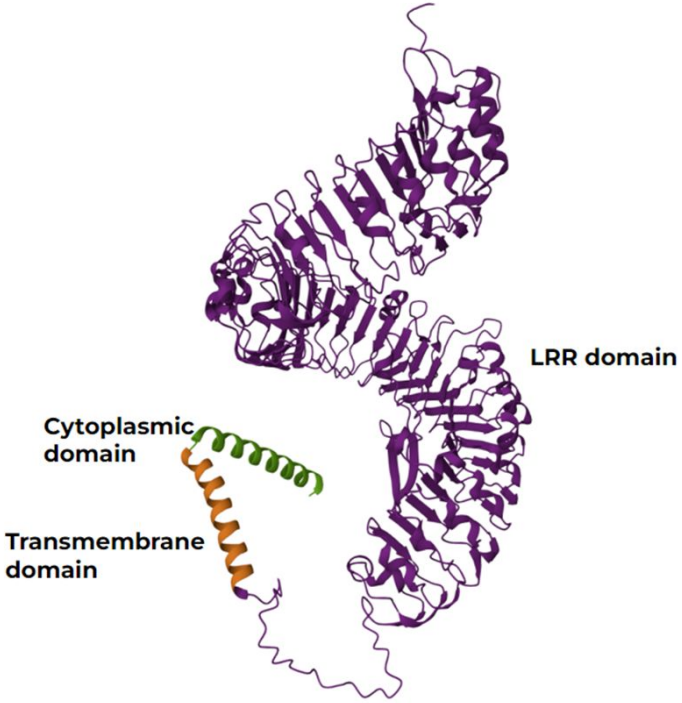


Entry	Chain	RMSD	TM-score	Identity	Equivalent Residues	Sequence Length	Modelled Residues
AvrRiv6_B04 SP free.pdb	A	-	-	-	-	75	75
6R5J	A	2.77	0.52	10%	54	68	67

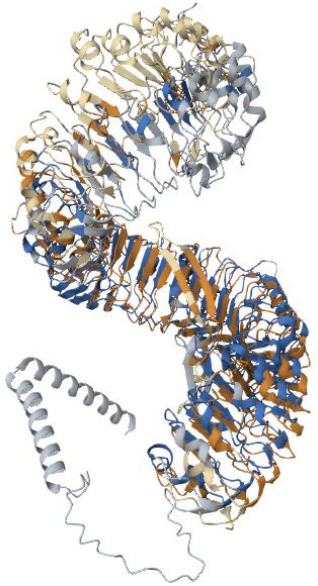
E = Coil ; H = Helix ; L=Beta sheet

Rvi6 is similar to RXEG1 in sequence, but also in structure

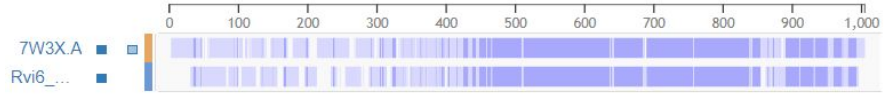
The 3D structure of Rvi6 was determined using AlphaFold2 Multimer V3



recycle = 20 pLDDT=90.6 pTM=0.791



Rvi6 aligned with RXEG1 using PDB align



Entry	Chain	RMSD	TM-score	Identity	Equivalent Residues	Sequence Length	Modelled Residues
7W3X	A	-	-	-	-	934	893
Rvi6_without_sp_unrelaxed_rank_001_alphafold2_multimer_v3_20_recyclings_on genotoul_server.pdb	A	5.1	0.74	31%	559	952	952

Does Rvi6 interact with AvrRvi6 ?

Through AlphaFold2 Multimer V3, the modeling of the complex Rvi6/AvrRvi6_B04 was done

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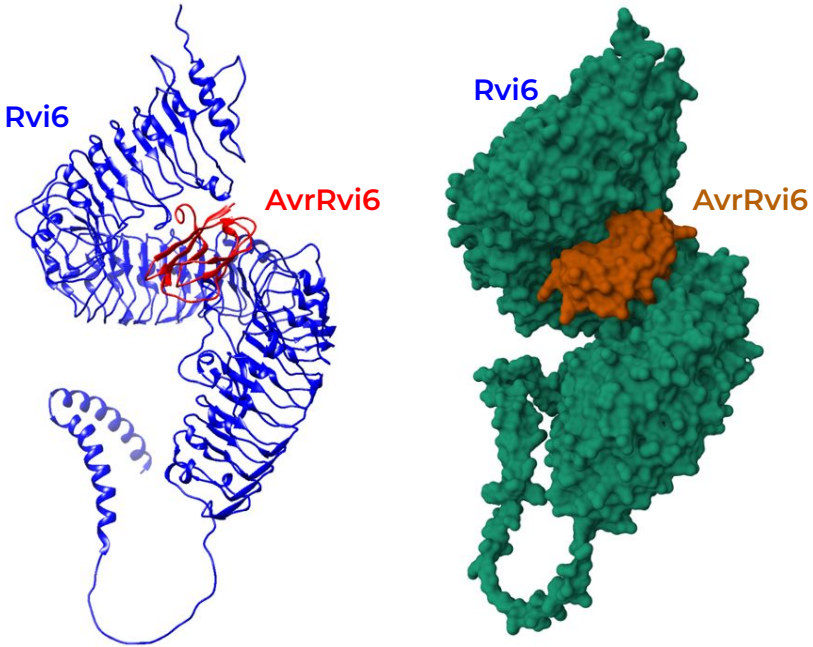

recycle=13 pLDDT=82.9 pTM=0.765 ipTM=0.472

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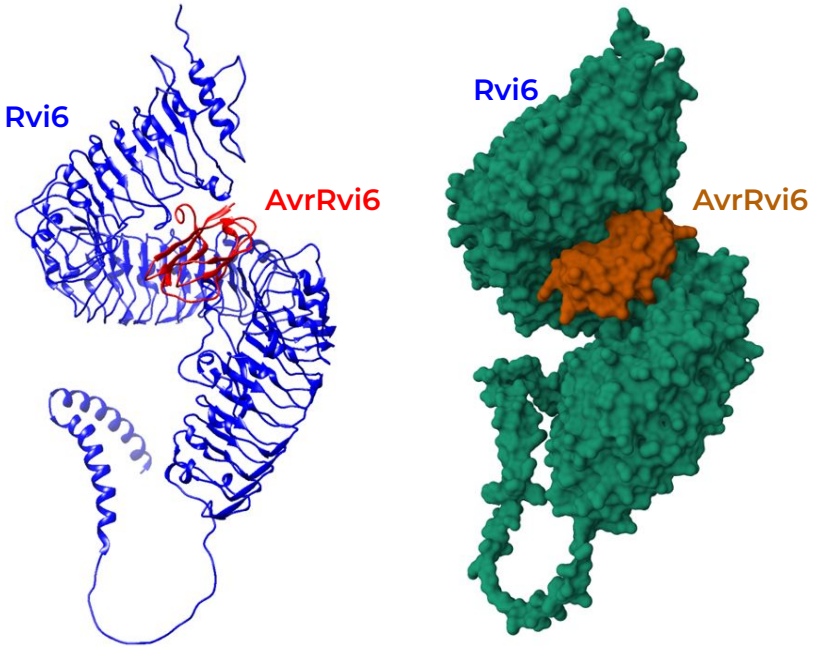

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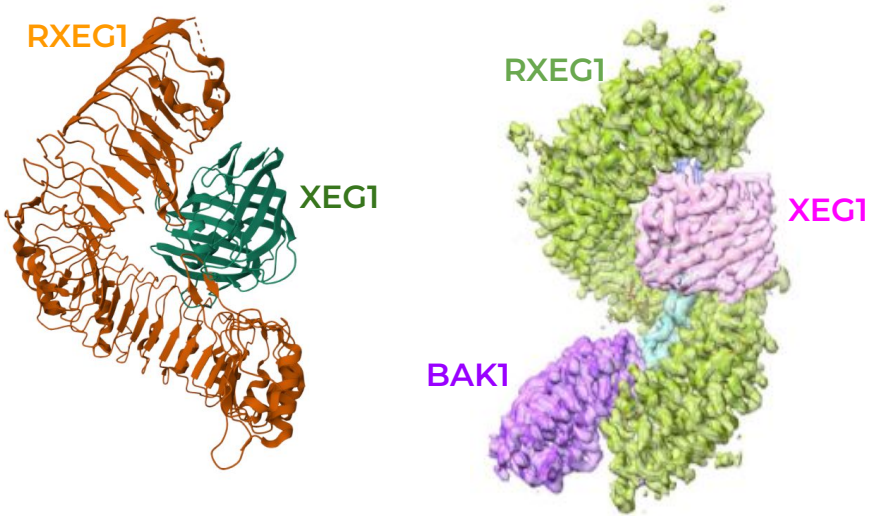
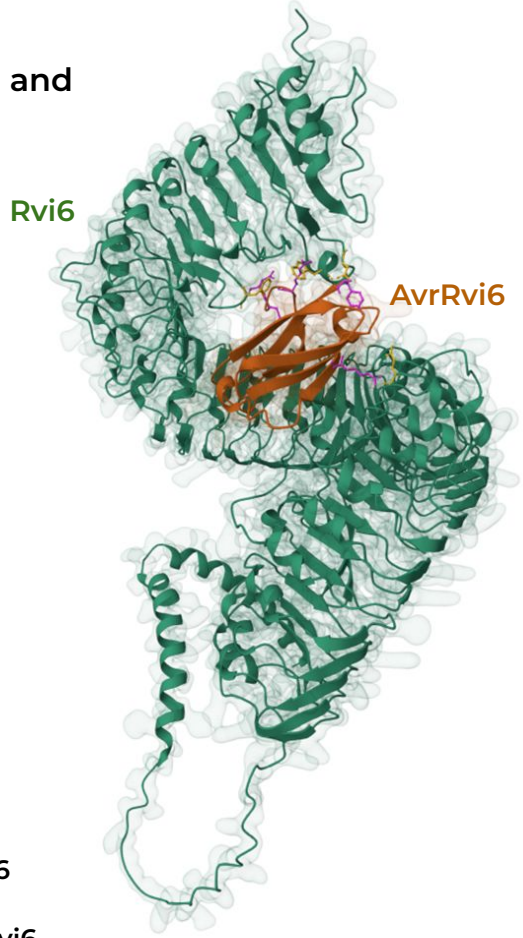


Complex structure solved through electron microscopy

(Sun *et al.*, 2022)

Rvi6/AvrRvi6 interaction sites prediction

Through complex protein modeling and the use of Chimera and PDB viewer



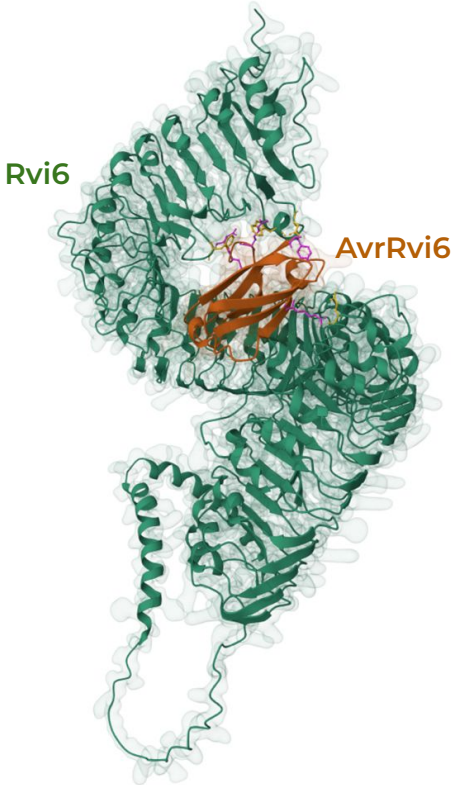
recycle=13 pLDDT=82.9 pTM=0.765 ipTM=0.472

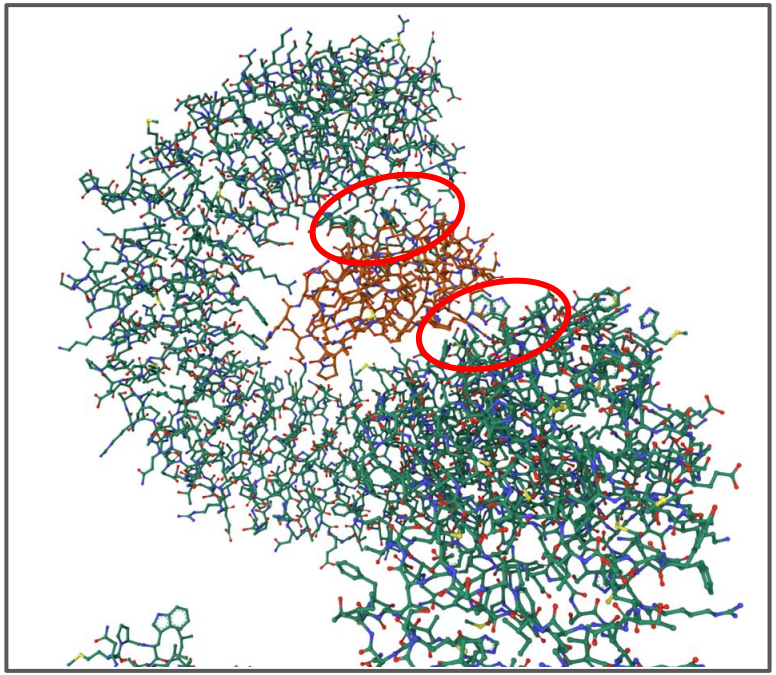
Rvi6	AvrRvi6
ASN 72	SER 2
	TYR 1
GLN 575	ARG 47
PHE 146	ASN 75
	CYS 74
	ARG 71
PHE 733	TYR 50
PHE 74	ARG 71
SER 599	ARG 47
TRP 623	ARG 49
	VAL 23
TRP 69	ARG 71
	VAL 17
TYR 121	ASN 75
TYR 147	THR 15
	GLY 16
TYR 66	PRO 70

Pink : AvrRvi6 residues interacting with Rvi6

Yellow : Rvi6 residues interacting with AvrRvi6

Rvi6/AvrRvi6 interaction sites predictions are similar to the ones between RXEG1/XEG1





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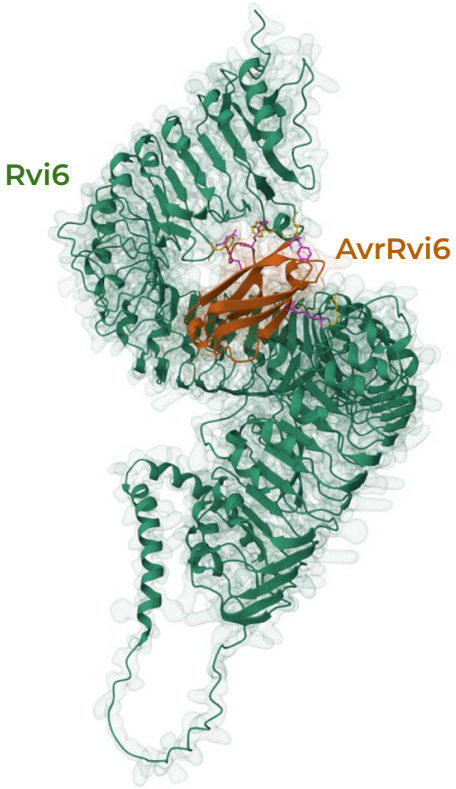

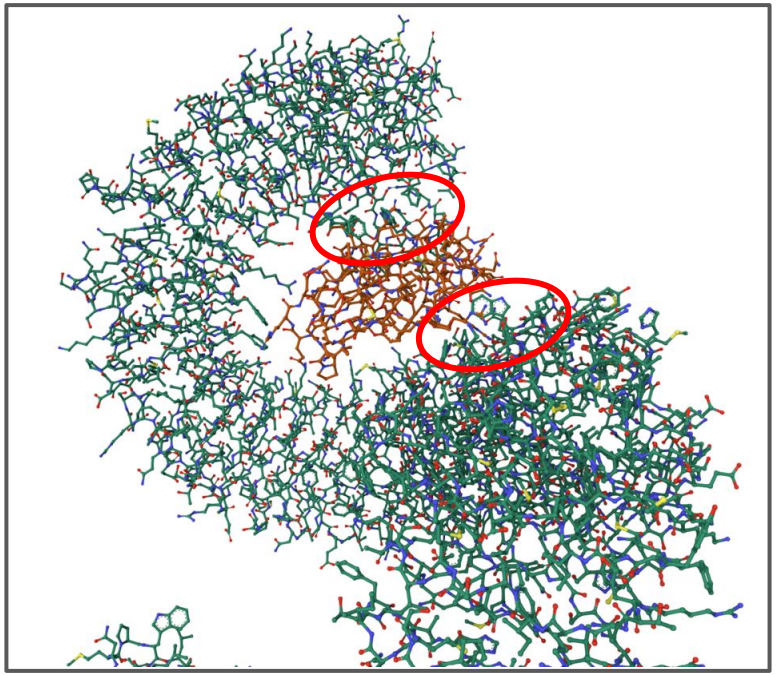

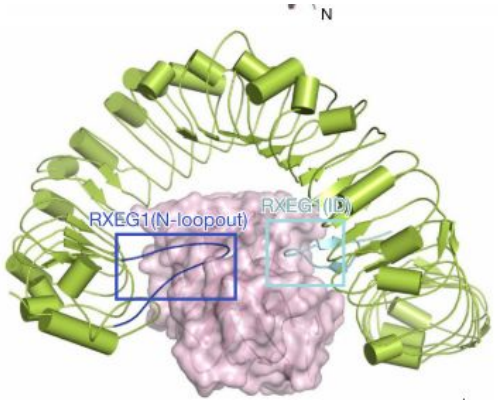

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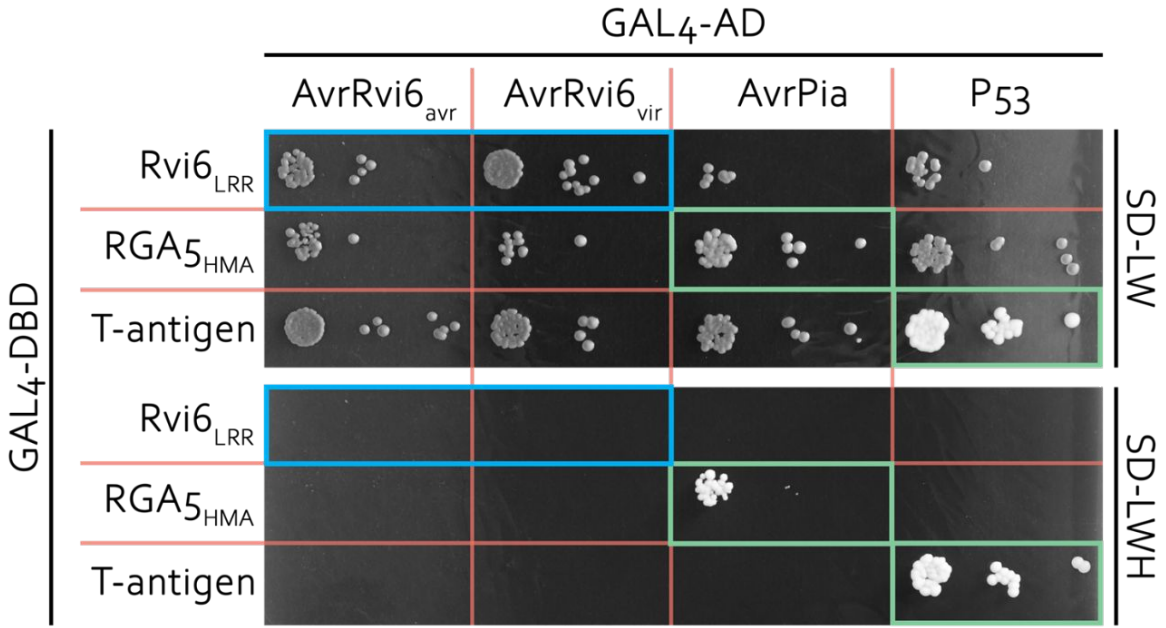
Yellow : Rvi6 residues interacting with AvrRvi6

Rvi6/AvrRvi6 do not interact in yeast (double hybrid method)

Full length LRRs are known to be misfolded in the nucleus of yeast

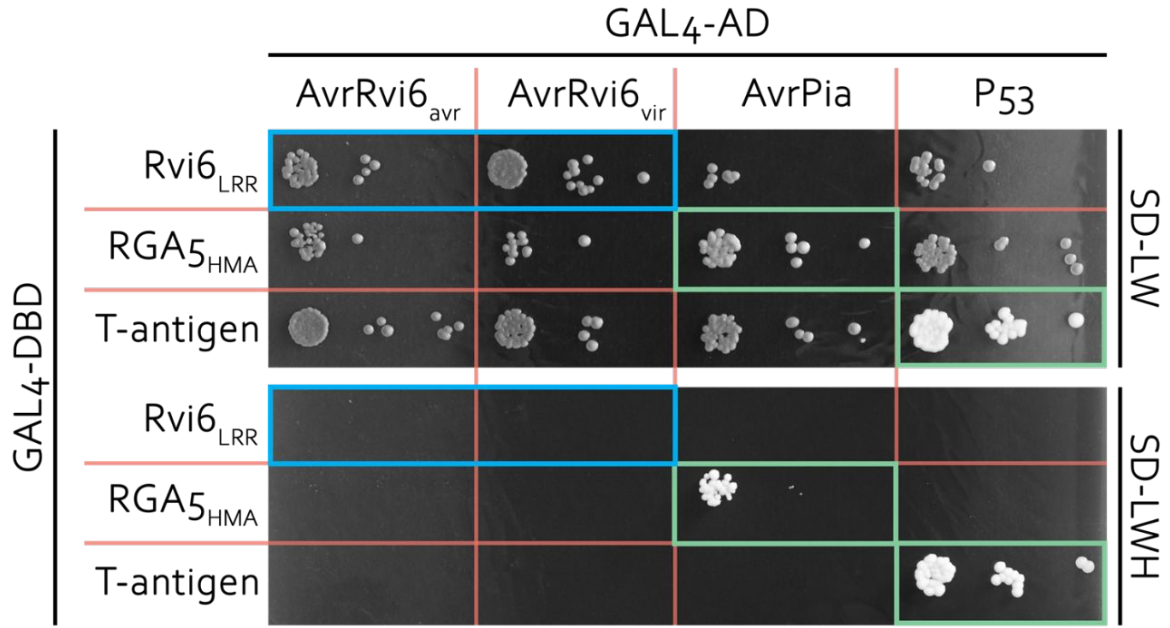


(Ilona Pires, master's internship 2023)

Rvi6/AvrRvi6 do not interact in yeast (double hybrid method)

Full length LRRs are known to be misfolded in the nucleus of yeast

Currently, parts of the LRR domain are being cloned based on the complex protein modeling
+
Currently generating constructs to test in *N. benthamiana* by CoIP



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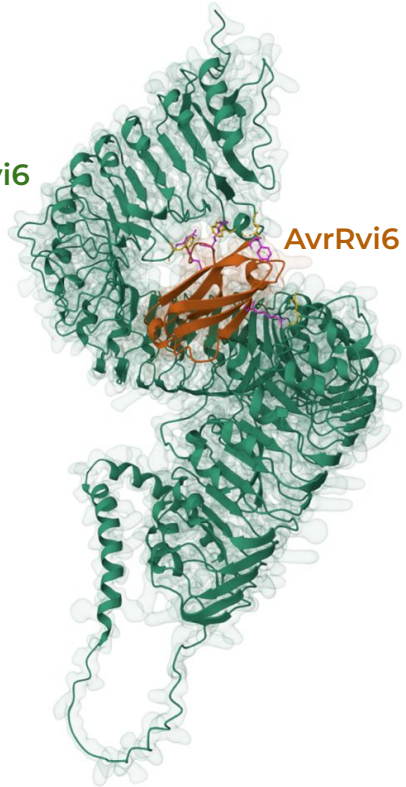
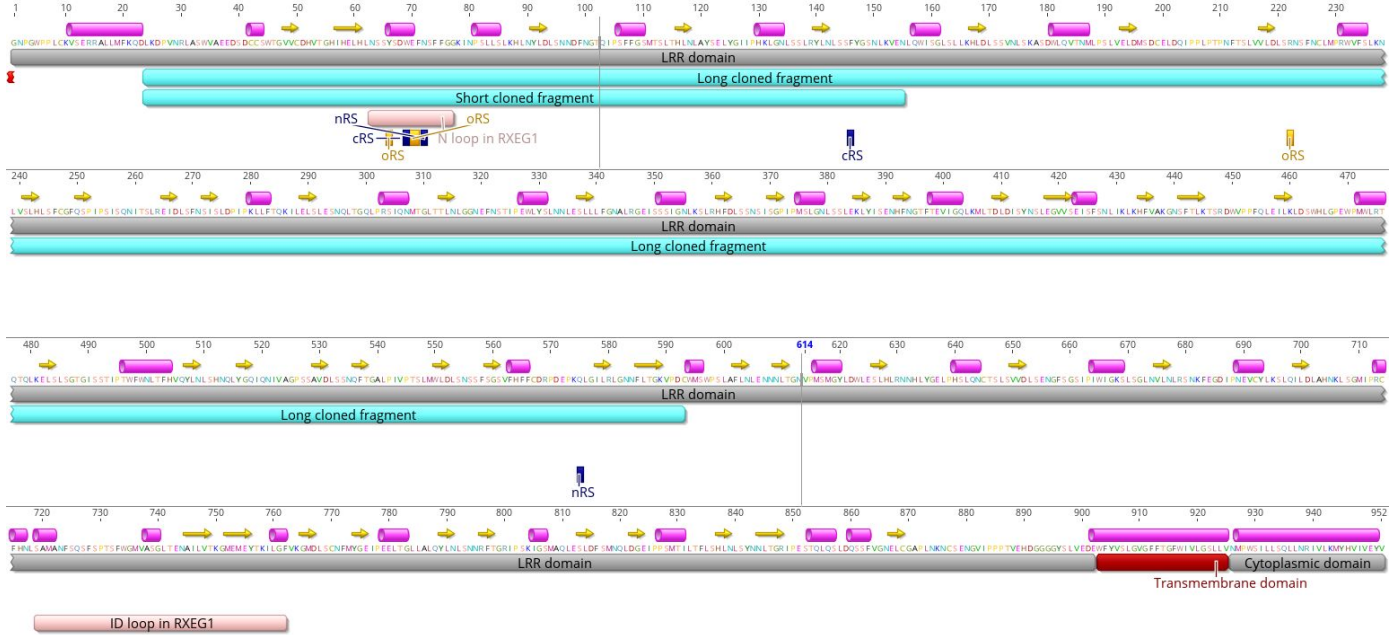
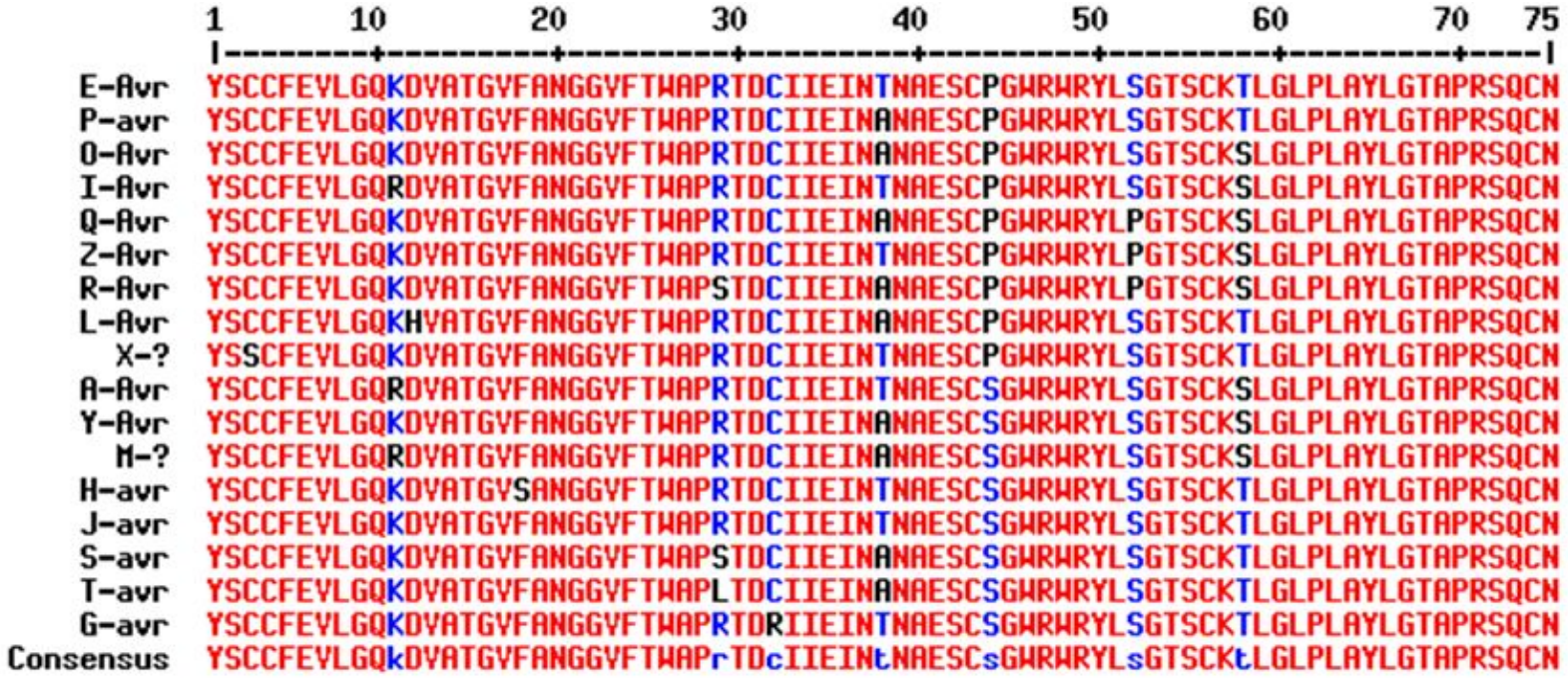




recycle=13 pLDDT=82.9 pTM=0.765 ipTM=0.472

AvrRvi6 allelic diversity and recognition escaping

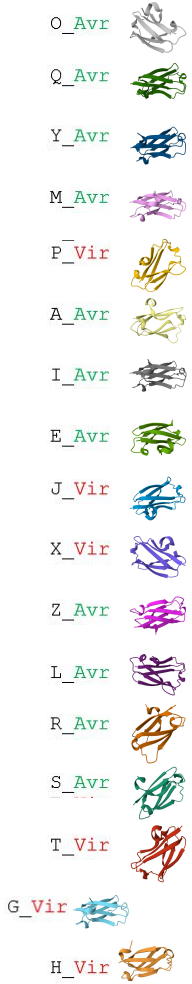
A multiple sequance alignement of all the known proteic AvrRvi6 alleles was made (using multalin)



A change in a single residue can cause a shift from avirulence to virulence

Small changes in structure can cause a virulence shift

- All known proteic alleles of AvrRvi6 were modeled using AlphaFold2



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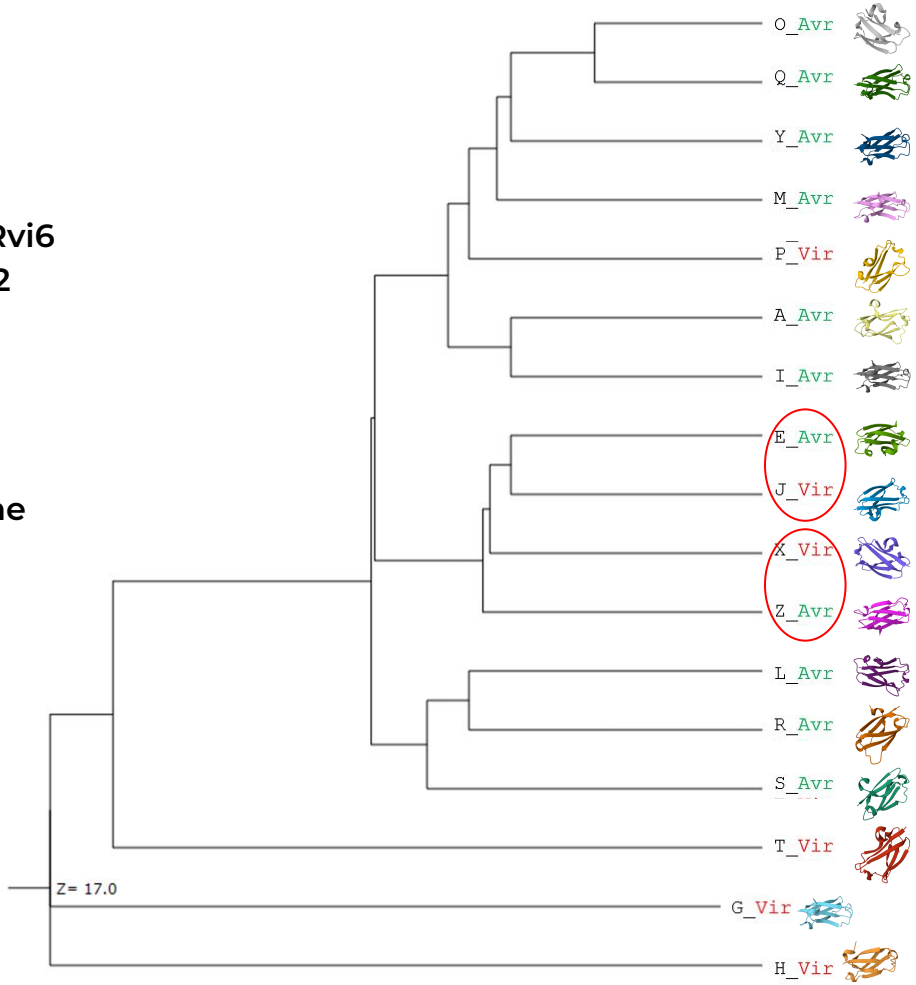
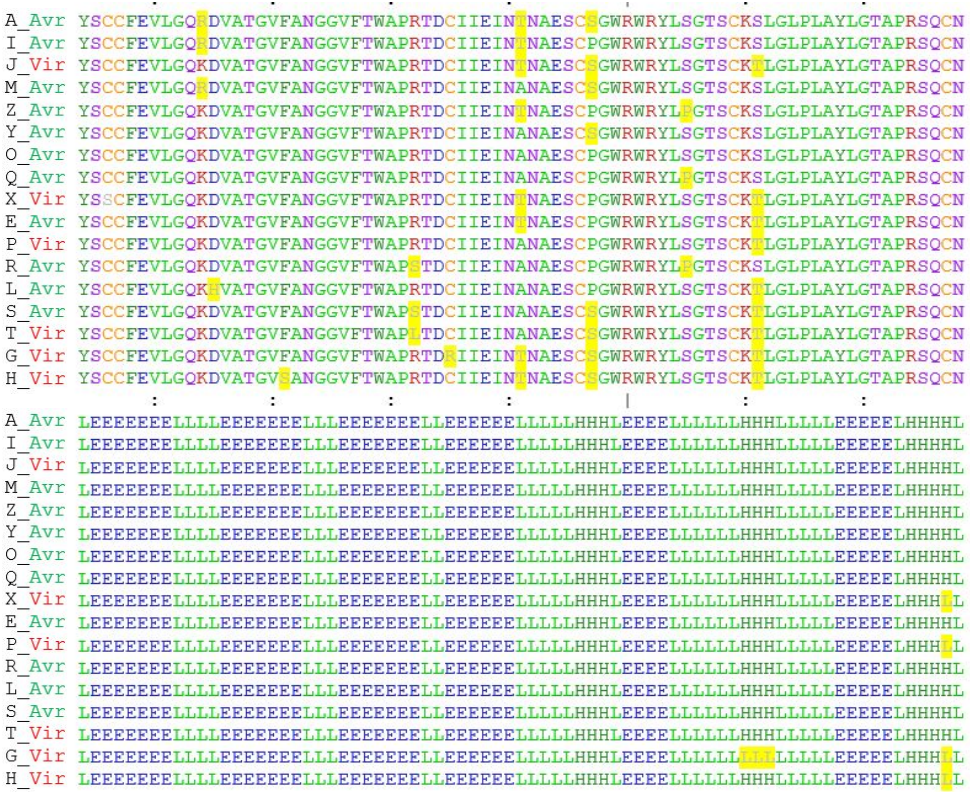
- All known proteic alleles of AvrRvi6 were modeled using AlphaFold2
- Using DALI, a dendrogram of the AvrRvi6 protein alleles was generated



Structural changes appear exclusively in virulent avrRvi6

A multiple structure alignement of all the known proteic AvrRvi6 alleles was made (using DALI)



Sites highlighted in yellow show polymorphism

E = Coil ; H = Helix ; L=Beta sheet

a → virulent allele ; A → Avirulent allele

Most structural changes are located in interaction sites

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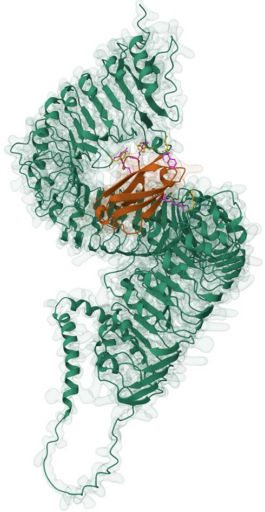

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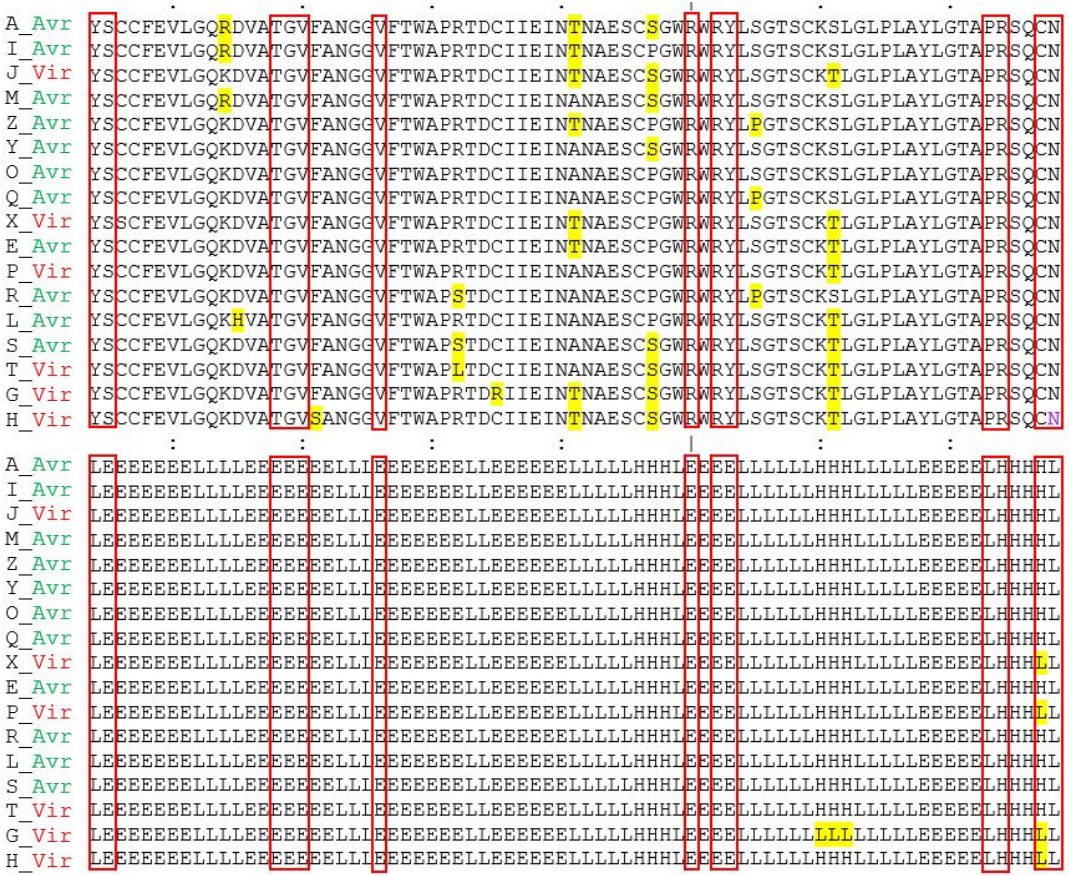
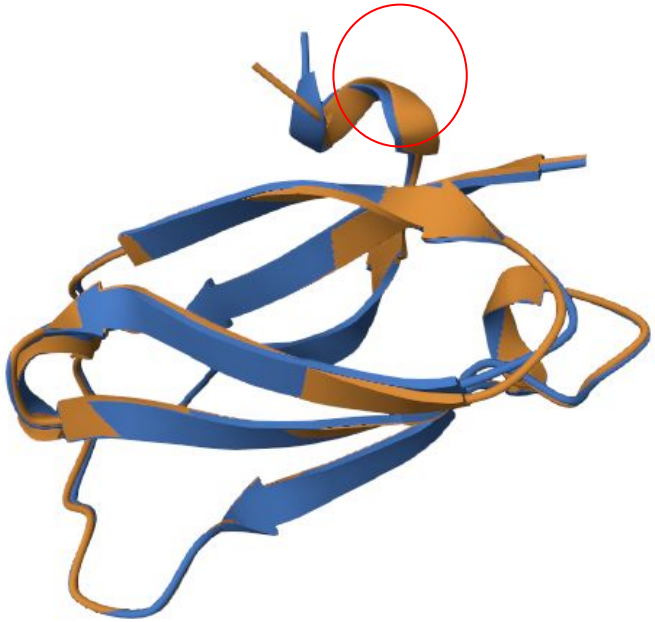
	CYS 74
	ARG 71
PHE 733	TYR 50
PHE 74	ARG 71
SER 599	ARG 47
TRP 623	ARG 49
	VAL 23
TRP 69	ARG 71
	VAL 17
TYR 121	ASN 75
TYR 147	THR 15
	GLY 16
TYR 66	PRO 70



The only alleles different structurally, are virulent

- The only structural changes detected are located in the C-ter extremity of the AvrRvi6 proteins in the virulent alleles.

  The change is a shortening of a helix.

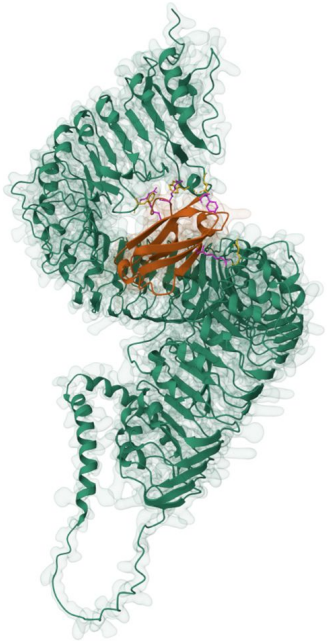


Structural alignment of AvrRvi6_H (virulent) with AvrRvi6_A (avirulent)

Entry	Chain	RMSD	TM-score	Identity	Equivalent Residues	Sequence Length	Modelled Residues
avrRvi6_H_noSP.pdb	A	-	-	-	-	75	75
AvrRvi6_B04_A_noSP.pdb	A	0.52	0.98	96%	75	75	75

Summary

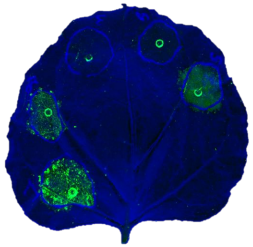
- A resistance protein and effector pair has been identified : Rvi6/AvrRvi6
- The interaction between Rvi6 and AvrRvi6 can be reproduced in a heterologous system : *N. benthamiana*
- AvrRvi6 is a member of the MAX structural family but is apoplastic unlike the others
- Protein and protein complex modeling allows a structure function analysis, all whilst using the available natural diversity



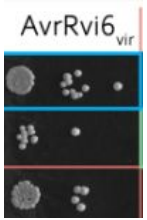
Perspectives

Rvi6 and AvrRvi6 natural diversity will be explored through exploration of mutation impact on interaction through :

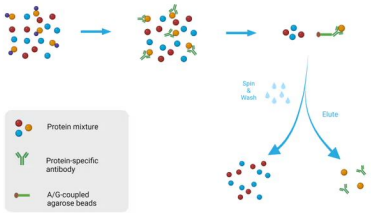
-Expression in *N. benthamiana*



-Development of yeast-two-hybrid tests



- CoIP



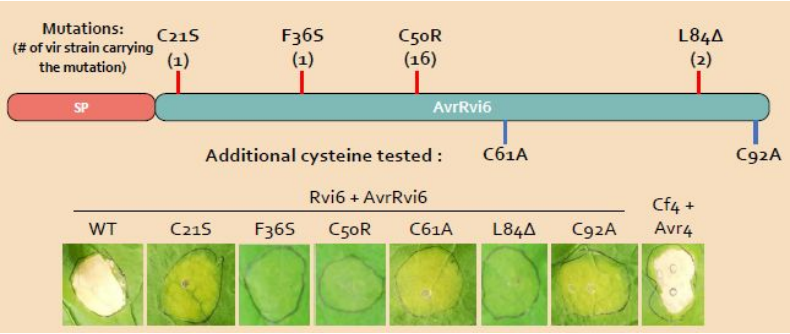
This work can contribute to rational engineering of immune receptors to broaden their recognition spector

Thank you for your attention !

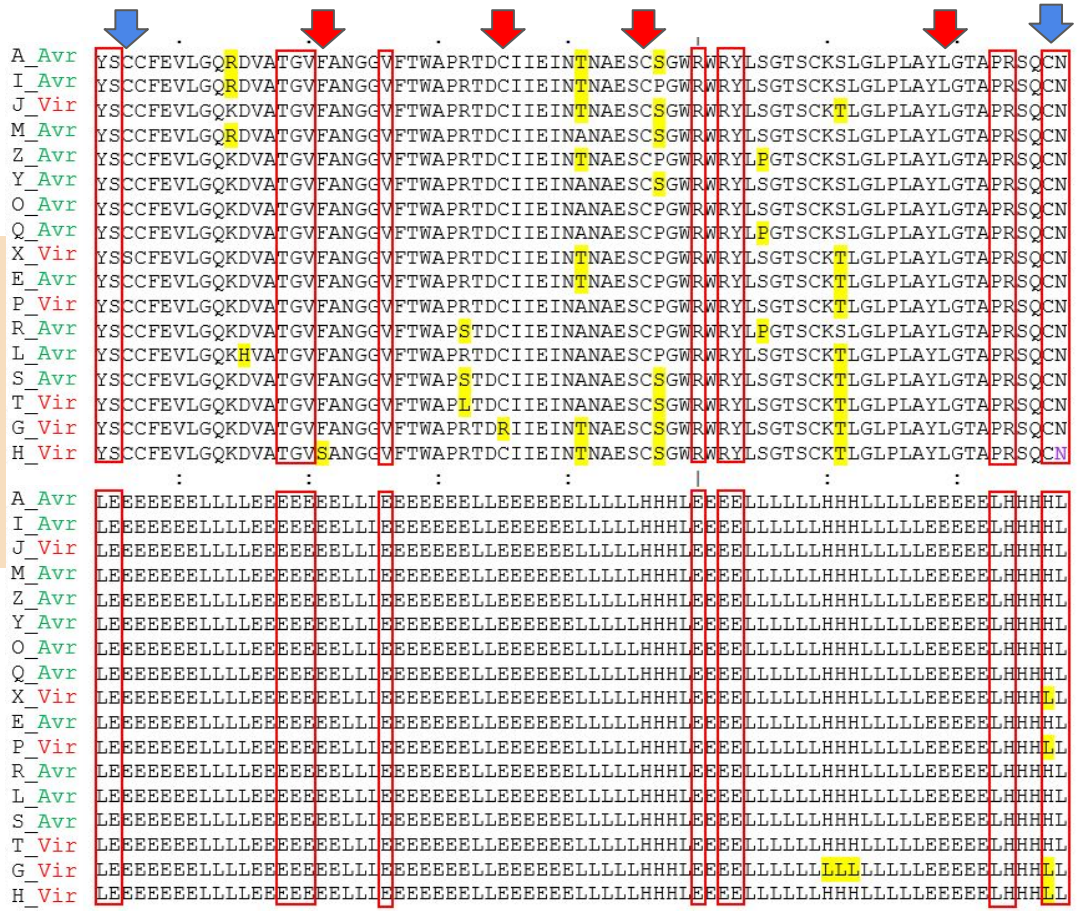
A special thanks goes the EcoFun team and our collaborators (S. Kamoun, S. Cesari, T. Kroj and C. Mesarich)

Certain mutations cause AvrRvi6 recognition loss in *N. benthamiana*

Conserved C residues were mutated on avirulent effector and generated loss or weakening of HR phenotypes on *N. benthamiana*



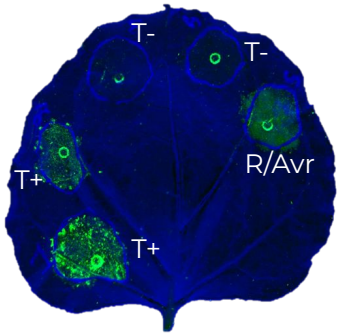
Alice Vassilère, Mélanie Sannier



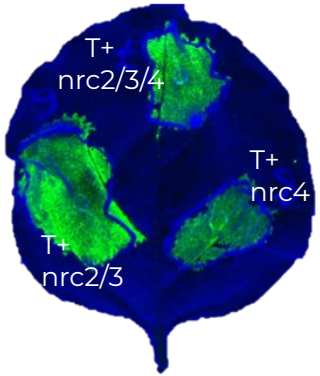
Do NRC2/3/4 have a role in the resistance to isolates carrying AvrRvi6 ?

Infiltration			Resulting reaction		
			HR	Very weak HR	No HR
T+	Cf4_A	Avr4_A	On all		
T+	Cf4_N	Avr4_N	Everything but mut 6	*nrc2/3* mut 6	
T-	Cf4_N	AvrRvi6 B04			On all
T-	Rvi6	avrRvi6_1180			On all
R/Avr	Rvi6	AvrRvi6 B04	*nrc4*	*nrc2/3*	
			WT NRC	*nrc2/3/4*	
			WT Angers		
T+nrc2/3	Pto	AvrPto	WT Angers		*nrc2/3*
T+ nrc2/3/4	Rx	CP	WT Angers		*nrc2/3/4*
T+ nrc4	Rpiblb2	Avrblb2	WT Angers		
			nrc4		

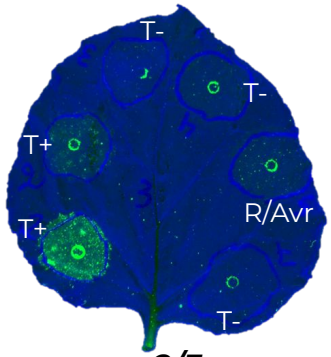
Transient expression in *N. benthamiana*



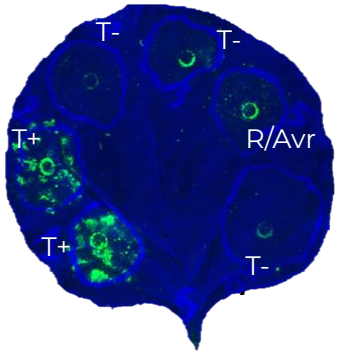
Wild type



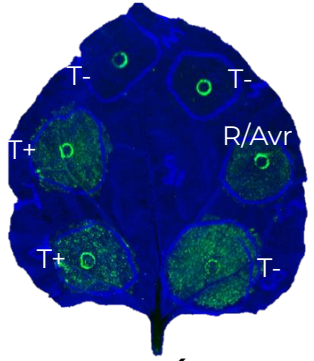
Wild type



nrc2/3



nrc2/3/4

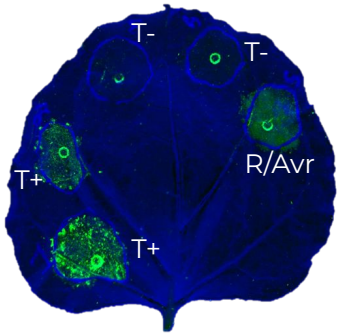


nrc4

Do NRC2/3/4 have a role in the resistance to isolates carrying AvrRvi6 ?

Infiltration			Resulting reaction		
			HR	Very weak HR	No HR
T+	Cf4_A	Avr4_A	On all		
T+	Cf4_N	Avr4_N	Everything but mut 6	*nrc2/3* mut 6	
T-	Cf4_N	AvrRvi6 B04			On all
T-	Rvi6	avrRvi6_1180			On all
R/Avr	Rvi6	AvrRvi6 B04	*nrc4*	*nrc2/3*	
			WT NRC	*nrc2/3/4*	
			WT Angers		
T+nrc2/3	Pto	AvrPto	WT Angers		*nrc2/3*
T+ nrc2/3/4	Rx	CP	WT Angers		*nrc2/3/4*
T+ nrc4	Rpiblb2	Avrblb2	WT Angers		
			nrc4		

Transient expression in *N. benthamiana*



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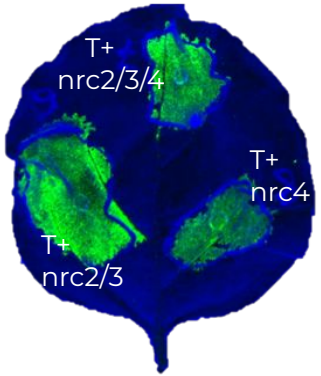
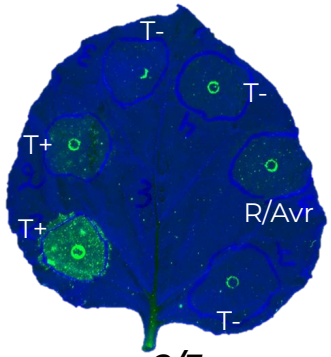
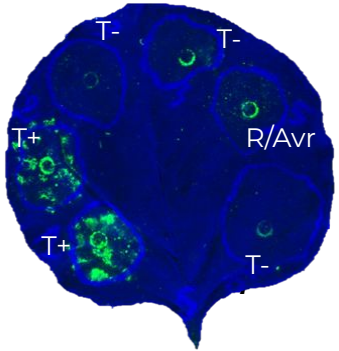


Wild type

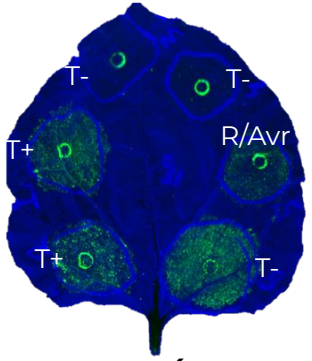
- Rvi6/AvrRvi6 recognition does not depend on NRC4 (or the mutant plant is still expressing NRC4).
- NRC2 and NRC3 seem to have an important role in the immune reaction against isolates carrying AvrRvi6



nrc2/3



nrc2/3/4

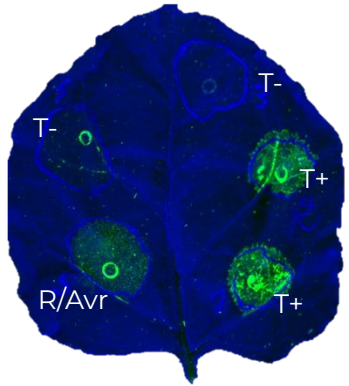


nrc4

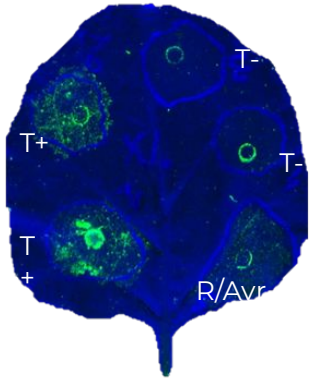
Do SOBIR1 and BAK1 have a role in the resistance to isolates carrying AvrRvi6 ?

Infiltration			Resulting reaction		
			HR	Very weak HR	No HR
T+	Cf4_A	Avr4_A	*bak1*		*sobir1*
			WT SOBIR1		
			WT Angers		
T+	Cf4_N	Avr4_N	*bak1*	*sobir1*	
			WT SOBIR1		
			WT Angers		
T-	Cf4_N	AvrRvi6 B04			On all
T-	Rvi6	avrRvi6_1180			On all
R/Avr	Rvi6	AvrRvi6 B04	WT SOBIR1	*bak1*	*sobir1*
			WT Angers		

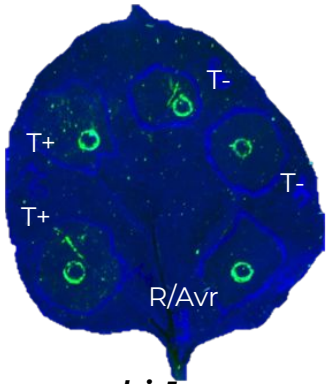
Transient expression in *N. benthamiana*



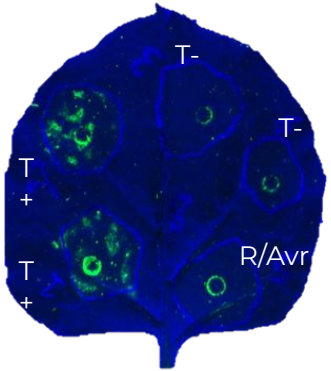
Wild type



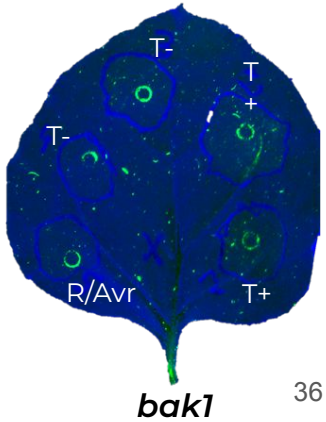
WT SOBIR1



sobir1



bak1

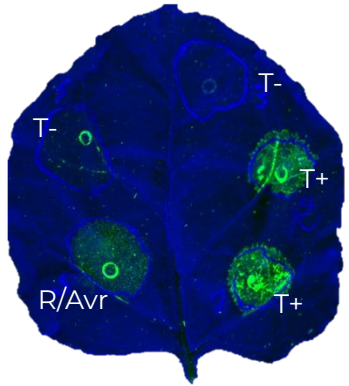


bak1

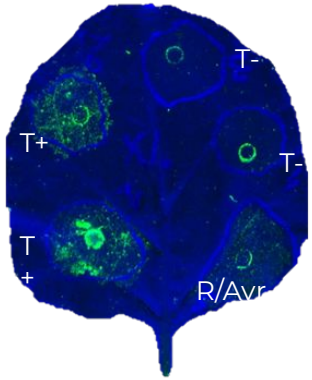
Do SOBIR1 and BAK1 have a role in the resistance to isolates carrying AvrRvi6 ?

Infiltration			Resulting reaction		
			HR	Very weak HR	No HR
T+	Cf4_A	Avr4_A	*bak1*		*sobir1*
			WT SOBIR1		
			WT Angers		
T+	Cf4_N	Avr4_N	*bak1*	*sobir1*	
			WT SOBIR1		
			WT Angers		
T-	Cf4_N	AvrRvi6 B04			On all
T-	Rvi6	avrRvi6_1180			On all
R/Avr	Rvi6	AvrRvi6 B04	WT SOBIR1	*bak1*	*sobir1*
			WT Angers		

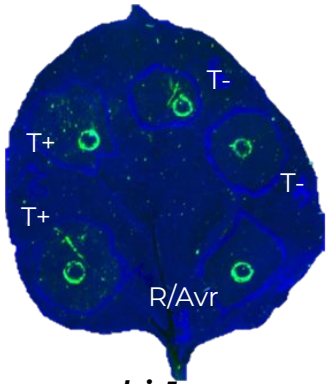
Transient expression in *N. benthamiana*



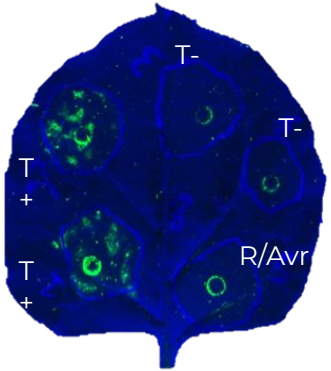
Wild type



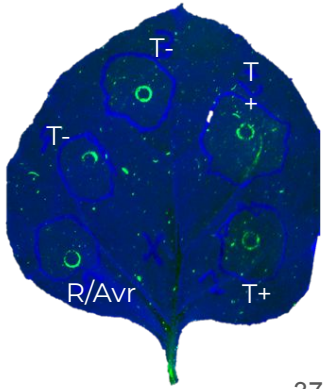
WT SOBIR1



sobir1



bak1



bak1

➡ BAK1 and SOBIR1 seem to have an important role in the immune reaction against isolates carrying AvrRvi6