Jean Chevaugeon days



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

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Team : EcoFun

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Venturia inaequalis, a fungal pathogen causing apple scab







(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

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



AvrRvi6 is a MAX effector

Rvi6 is an RLP (with a protein sequence similar to RXEG1



RXEG1's resolved structure

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



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RXEG1's resolved structure

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



Cf4 and Avr4 are positive HR controls.

Rvi6 recognition of AvrRvi6 can be replicated in N. benthamiana



Cf4 and Avr4 are positive HR controls.



AvrRvi6 HR is dependent on the secretion of the effector in the apoplast.

(SP = secretion Signal Peptide)







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



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)





- 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 The 3D structure of AvrRvi6 was determined using AlphaFold2



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

The 3D structure of AvrRvi6 was determined using AlphaFold2





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

-

		0	10	20	30	40	50	60	70	80
AvrRiv	•	1	SCCFEVES QRDV	AT GVF ANG	GVFTWAPH	DCILEINTN	NESCS GWRWIT	LSGTSCKSLG	LPLAYLGIAPH	SQCN
6R5J.A		-	GCSVELINS	USGCAR1N	SVINIGDNOD	RRWGVLA	VSSCG LSTT	N LP	SAN SLIDTO	CNA

Entry	Chain	RMSD	TM-score	Identity	Equivalent Residues	Sequence Length	Modelled Residues
AvrRiv6_B04 SP free.pdb	A	-	-	-	1.51	75	75
6R5J	A	2.77	0.52	10%	54	68	67

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

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



genotoul server.pdb

Does Rvi6 interact with AvrRvi6?

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

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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



Rvi6	AvrRvi6		
ACN 72	SER 2		
ASN 72	TYR 1		
GLN 575	ARG 47		
	ASN 75		
PHE 146	CYS 74		
	ARG 71		
PHE 733	TYR 50		
PHE 74	ARG 71		
SER 599	ARG 47		
	ARG 49		
TKP 023	VAL 23		
	ARG 71		
IKP 09	VAL 17		
TYR 121	ASN 75		
TVD 147	THR 15		
116 147	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



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

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



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

Pink : AvrRvi6 residues interacting with Rvi6 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



(Ilona Pires, master's internship 2023)

Rvi6/AvrRvi6 interaction sites prediction



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)

	1	10	20	30	40	50	60	70	75
	I	+	+	+	+	+	+	+-	1
E-Avr	YSCCFI	EYLGQKDYA	TGYFANGGYF	THAPRTDCI	EINTNAESCP	GHRHRYLSGT	SCKTLGLPLAY	LGTAPR	SQCN
P-avr	YSCCFI	EYLGQ <mark>k</mark> dya	TGYFANGGYF	THAPRTDCI	EINANAESCP	GHRHRYLSGT	SCKTLGLPLAY	LGTAPR	SQCN
0-Avr	YSCCFI	EYLGQKDYA	TGYFANGGYF	THAPRTDCI	EINANAESCP	GHRHRYLSGT	SCKSLGLPLAY	LGTAPR	SQCN
I-Avr	YSCCFI	EYLGQRDYA	TGYFANGGYF	THAPRTDCI	EINTNAESCP	GHRHRYLSGT	SCKSLGLPLAY	LGTAPR	SQCN
Q-Avr	YSCCFI	EYLGQKDYA	TGYFANGGYF	THAPRTDCI	EINANAESCP	GHRHRYLPGT	SCKSLGLPLAY	LGTAPR	SQCN
Z-Avr	YSCCFI	EYLGQKDYA	TGYFANGGYF	THAPRTDCI	EINTNAESCP	GHRHRYLPGT	SCKSLGLPLAY	LGTAPR	SQCN
R-Avr	YSCCFI	EYLGQKDYA	TGYFANGGYF	THAPSTOCI]	EINANAESCP	GHRHRYLPGT	SCKSLGLPLAY	LGTAPR	SQCN
L-Avr	YSCCF	EYLGQKHYA	TGYFANGGYF	THAPRTDCI	EINANAESCP	GHRHRYLSGT	SCKTLGLPLAY	LGTAPR	SQCN
X-?	YSSCFI	EYLGQKDYA	TGYFANGGYF	THAPRTDCI	EINTNAESCP	GHRHRYLSGT	SCKTLGLPLAY	LGTAPR	SQCN
A-Avr	YSCCFI	EYLGQRDYA	TGYFANGGYF	THAPRTDCI	EINTNAESCS	GHRHRYLSGT	SCKSLGLPLAY	LGTAPR	SQCN
Y-Avr	YSCCFI	EYLGQKDYA	TGYFANGGYF	THAPRTDCI	EINANAESCS	GHRHRYLSGT	SCKSLGLPLAY	LGTAPR	SQCN
H-?	YSCCFI	EYLGQRDYA	TGYFANGGYF	THAPRTDCI	EINANAESCS	GHRHRYLSGT	SCKSLGLPLAY	LGTAPR	SQCN
H-avr	YSCCFI	EYLGQKDYA	TGYSANGGYF	THAPRTDCI	EINTNAESCS	GHRHRYLSGT	SCKTLGLPLAY	LGTAPR	SQCN
J-avr	YSCCFI	EYLGQKDYA	TGYFANGGYF	THAPRTDCI	EINTNAESCS	GHRHRYLSGT	SCKTLGLPLAY	LGTAPR	SQCN
S-avr	YSCCFI	EYLGQKDYA	TGYFANGGYF	THAPSTOCI	EINANAESCS	GHRHRYLSGT	SCKTLGLPLAY	LGTAPR	SQCN
T-avr	YSCCFI	EYLGQKDYA	TGYFANGGYF	THAPL TOCI	EINANAESCS	GHRHRYLSGT	SCKTLGLPLAY	LGTAPR	SQCN
G-avr	YSCCFI	EYLGQKDYA	TGYFANGGYF	THAPRTORIJ	EINTNAESCS	GHRHRYLSGT	SCKTLGLPLAY	LGTAPR	SQCN
Consensus	YSCCF	EYLGQkDYA	TGYFANGGYF	THAPrTDcI	EINTNAESCS	GHRHRYLSGT	SCKELGLPLAY	LGTAPR	SQCN

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



Small changes in structure can cause a virulence shift



Structural changes appear exclusively in virulent avrRvi6

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

```
A AVY YSCCFEVLGO DVATGVFANGGVFTWAPRTDCIIEIN NAESC GWRWRYLSGTSCKSLGLPLAYLGTAPRSQCN
I Avr ysccfevlgo Dvatgvfanggvffwaprtdciiein NAEscpgwrwryi
J Vir
      YSCCFEVLGOKDVATGVFANGGVFTWAPRTDCIIEINUNAESC
                                                   GWRWRYLSGTSCKTLGLPLAYLGTAPRSOCN
      YSCCFEVLGO DVATGVFANGGVFTWAPRTDCIIEINANAESCSGWRWRYLSGTSCKSLGLPLAYLGTAPRSOCN
Z Avr
          FEVLGOKDVATGVFANGGVFTWAPRTDCIIEIN NAESCPGWRWRYLPGTSCKSLGLPLAY
Y Avr
                               TWAPRTDCIIEINANAESC<mark>S</mark>GWRWRYLSGTS(
O Avr
          FEVIGORDVATGVFANGGVF
                                   PRTDCTTEINANAESCPGWRWRYLSGTS
                                       IIEINANAESCPGWRWRYLPGTSCKSLGLPLAYLGTAP
      YSSCFEVLGOKDVATGVFANGGVFTWAPRTDCIIEINUNAESCPGWRWRYLSGTSCKULGLPLAYLGTAPRSOCN
                                WAPRTDCIIEINUNAESCPGWRWRYLSGTSCK
P Vir
                                       IIEINANAESCPGWRWRYLSGTSCK
                                                   PGWRWRYLPGTSCKSLGLE
T Vir
      YSCCFEVLGQKDVATGVFANGGVFTWAP_TDCIIEINANAESC
                                                   GWRWRYLSGTSCK
G Vir YSCCFEVLGOKDVATGVFANGGVFTWAPRTD IIEIN NAESC
                                                   GWRWRYLSGTSCK
H Vir
      YSCCFEVLGOKDVATGV ANGGVFTWAPRTDCIIEIN NAESC GWRWRYLSGTSCK
J Vir
M Avr
Z Avr
Y Avr
ΤV
G V:
Η
                                                 J.HHHLEEFELLLLLLHHHLLLLEFEFELHHH
               ILLIEFFFFFLLIFFFFFFLLFFFFFLL
```

Sites highlighted in yellow show polymorphism

E = Coil ; H = Helix ; L=Beta sheet a \rightarrow virulent allele ; A \rightarrow Avirulent allele

Most structural changes are located in interaction sites

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



Rvi6	AvrRvi6		
	SER 2		
ASN 72	TYR 1		
GLN 575	ARG 47		
	ASN 75		
PHE 146	CYS 74		
	ARG 71		
PHE 733	TYR 50		
PHE 74	ARG 71		
SER 599	ARG 47		
	ARG 49		
TKP 023	VAL 23		
	ARG 71		
TRP 09	VAL 17		
TYR 121	ASN 75		
TVD 147	THR 15		
ITK 147	GLY 16		
TYR 66	PRO 70		

			•		_			
A_Avr	YSCCFEVLGQRDVA	TGVFANG	GVFTWAPRTDC	IIEIN <mark>T</mark> NAESC <mark>S</mark>	GWRWRY	LSGTSCKS	LGLPLAYLGTAPRS	SQCN
I_Avr	YSCCFEVLGQRDVA	TGVFANG	GVFTWAPRTDC	IIEINTNAESCP	GWRWRY	LSGTSCKS	LGLPLAYLGTAPRS	SQCN
J_Vir	YSCCFEVLGQKDVA	TGVFANG	GVFTWAPRTDC	IIEIN <mark>T</mark> NAESC <mark>S</mark>	GWRWRY	LSGTSCKT	LGLPLAYLGTAPRS	SQCN
M_Avr	YSCCFEVLGQRDVA	TGVFANG	GVFTWAPRTDC	IIEINANAESCS	GWRWRY	LSGTSCKS	LGLPLAYLGTAPRS	SQCN
Z_Avr	YSCCFEVLGQKDVA	TGVFANG	GVFTWAPRTDC	IIEINTNAESCP	GWRWRY	LPGTSCKS	lglplaylgta <mark>pr</mark> s	SQCN
Y_Avr	YSCCFEVLGQKDVA	TGVFANG	GVFTWAPRTDC	IIEINANAESCS	GWRWRY	LSGTSCKS	lglplaylgta <mark>pr</mark> s	SQCN
O_Avr	YSCCFEVLGQKDVA	TGVFANG	GVFTWAPRTDC	IIEINANAESCP	GWRWRY	LSGTSCKS	lglplaylgta <mark>pr</mark> s	SQCN
Q_Avr	YSCCFEVLGQKDVA	TGVFANG	GVFTWAPRTDC	IIEINANAESCP	GWRWRY	LPGTSCKS	LGLPLAYLGTAPRS	SQCN
X_Vir	YSSCFEVLGQKDVA	TGVFANG	GVFTWAPRTDC	IIEIN <mark>T</mark> NAESCP	GWRWRY	LSGTSCKT	lglplaylgtaprs	SQCN
E_Avr	YSCCFEVLGQKDV	TGVFANG	GVFTWAPRTDC	IIEIN <mark>T</mark> NAESCP	GWRWRY	LSGTSCK <mark>T</mark> I	lglplaylgtaprs	SQCN
P_Vir	YSCCFEVLGQKDVA	TGVFANG	GVFTWAPRTDC	IIEINANAESCP	GWRWRY	LSGTSCKT	lglplaylgta <mark>pr</mark> s	SQCN
R_Avr	YSCCFEVLGQKDVA	TGVFANG	GVFTWAP <mark>S</mark> TDC	IIEINANAESCP	GWRWRY	LPGTSCKS	lglplaylgtaprs	SQCN
L_Avr	YSCCFEVLGQK <mark>H</mark> VF	TGVFANG	GVFTWAPRTDC	IIEINANAESCP	GWRWRY	LSGTSCKT	LGLPLAYLGTAPRS	SQCN
S_Avr	YSCCFEVLGQKDVA	TGVFANG	GVFTWAP <mark>S</mark> TDC	IIEINANAESC <mark>S</mark>	GWRWRY	LSGTSCKT	LGLPLAYLGTAPRS	SQCN
T_Vir	YSCCFEVLGQKDVA	TGVFANG	GVFTWAPLTDC	IIEINANAESC <mark>S</mark>	GWRWRY	LSGTSCKT	LGLPLAYLGTAPRS	SQCN
G_Vir	YSCCFEVLGQKDVA	TGVFANG	GVFTWAPRTD <mark>R</mark>	IIEIN <mark>T</mark> NAESC <mark>S</mark>	GWRWRY	LSGTSCKT	LGLPLAYLGTAPRS	SQCN
H Vir	YSCCFEVLGQKDV#	TGVSANG	GVFTWAPRTDC	IIEINTNAESCS	GWRWRY	LSGTSCKT	LGLPLAYLGTAPRS	SQCN
200 T 100								
-	:		:	:	1		: 22	
A_Avr	: LEEEEEELLLEE	: EEEEELI	: I <mark>E</mark> EEEEELLE	: EEEEELLLLLHH	 HLEEEE	: LLLLLLHHI	: HLLLLEEEEE <mark>LH</mark> H	IHHL
A_Avr I_Avr	: LEEEEEEELLLEE LEEEEEELLLEE	: EEEEELI EEEEELI	: I <mark>E</mark> EEEEELLE IEEEEEELLE	: EEEEELLLLLHH EEEEELLLLLHH	 HLEEEE HLEEEE	: LLLLLLHHI LLLLLHHI	: HLLLLEEEEE <mark>LH</mark> H HLLLLEEEEE <mark>LH</mark> H	IHHL IHHL
A_Avr I_Avr J_Vir	: LESEEEEELLLEE LESEEEEELLLEE LESEEEEELLLEE	: EEEEELI EEEEELI EEEEELI	: I <mark>E</mark> EEEEEELLE IEEEEEELLE	: EEEEELLLLLHH EEEEELLLLLHH EEEEELLLLLHH	 HLEEEE HLEEEE HLEEEE	: LLLLLLHHI LLLLLHHI LLLLLLHHI	: HLLLLLEEEEE <mark>LHH</mark> HLLLLLEEEEELHH	IHHL IHHL IHHL
A_Avr I_Avr J_ <mark>Vir</mark> M_Avr	: LESEEEEELLLEE LESEEEEELLLEE LESEEEEELLLEE LESEEEEELLLEE	: EEEEELI EEEEELI EEEEELI EEEEELI	: IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE	: EEEEELLLLLHH EEEEELLLLLHH EEEEELLLLLHH	I HLEEEE HLEEEE HLEEEE	: LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI	: HLLLLLEEEEELHH HLLLLLEEEEELHH HLLLLLEEEEELHH	IHHL IHHL IHHL IHHL
A_Avr I_Avr J_Vir M_Avr Z_Avr	LE EEEEEELLLLEE LE EEEEEELLLLEE LE EEEEEELLLLEE LE EEEEEELLLLEE LE EEEEEELLLLEE	: EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI	: IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE	: EEEEELLLLLHH EEEEELLLLLHH EEEEELLLLLHH EEEEELLLLLHH EEEEELLLLLHH	I HLEEEE HLEEEE HLEEEE HLEEEE	: LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI	: HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH	IHHL IHHL IHHL IHHL IHHL
A_Avr I_Avr J_Vir M_Avr Z_Avr Y_Avr	LE EEEEEELLLLEE LE EEEEEELLLLEE LE EEEEEELLLLEE LE EEEEEELLLLEE LE EEEEEELLLLEE LE EEEEEELLLLEE	: EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI	: IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE	: EEEEELLLLLHH EEEEELLLLLHH EEEEELLLLLHH EEEEELLLLLHH EEEEELLLLLHH	I HLEEEE HLEEEE HLEEEE HLEEEE HLEEEE	: LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI	: HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH	IHHL IHHL IHHL IHHL IHHL IHHL
A_Avr I_Avr J_Vir M_Avr Z_Avr Y_Avr O_Avr	: LEEEEEEELLLEE LEEEEEELLLEE LEEEEEELLLEE LEEEEEE	: EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI	: IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE	: EEEEELLLLLHH EEEEELLLLLHH EEEEELLLLLHH EEEEELLLLLHH EEEEELLLLLHH EEEEELLLLLHH	I HLEEEE HLEEEE HLEEEE HLEEEE HLEEEE	: LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI	: HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH	IHHL IHHL IHHL IHHL IHHL IHHL IHHL
A_Avr I_Avr J_Vir M_Avr Z_Avr Y_Avr Q_Avr	: LE EEEEEELLLLEE LE EEEEEELLLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE	: EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI	IPEEEEELLE IPEEEEEELLE IPEEEEELLE IPEEEEELLE IPEEEEEELLE IPEEEEEELLE IPEEEEEELLE IPEEEEEELLE	: EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH	I HLEEEE HLEEEE HLEEEE HLEEEE HLEEEE HLEEEE	: LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI	: HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH	IHHL IHHL IHHL IHHL IHHL IHHL IHHL IHHL
A_Avr I_Avr J_Vir M_Avr Z_Avr Y_Avr Q_Avr X_Vir	: LE EEEEEELLLLEE LE EEEEEELLLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE	: EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI	IPEEEEELLE IEEEEEELLE IEEEEEELLE IEEEEEELLE IEEEEEELLE IEEEEEELLE IEEEEEELLE IEEEEEELLE IEEEEEELLE	: EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH	I HLEEEE HLEEEE HLEEEE HLEEEE HLEEEE HLEEEE HLEEEE	: LLLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI	: HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH	IHHL IHHL IHHL IHHL IHHL IHHL IHHL IHHL
A_Avr I_Avr J_Vir M_Avr Z_Avr Y_Avr O_Avr Q_Avr X_Vir E_Avr	: LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLEE	: EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI	IPEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE	: EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH	I HLEEEE HLEEEE HLEEEE HLEEEE HLEEEE HLEEEE HLEEEE HLEEEE	: LLLLLLHH LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI	: HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH	IHHL IHHL IHHL IHHL IHHL IHHL IHHL IHHL
A_Avr I_Avr J_Vir M_Avr Z_Avr Y_Avr O_Avr Q_Avr X_Vir E_Avr P_Vir	: LEEEEEELLLEE LEEEEEELLLEE LEEEEEELLLEE LEEEEEE	: EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI	: IPEEEEEELLE IPEEEEEELLE IPEEEEEELLE IPEEEEEELLE IPEEEEEELLE IPEEEEEELLE IPEEEEEELLE IPEEEEEELLE IPEEEEEELLE IPEEEEEELLE	: EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH	I HIEEEEE HIEEEEE HIEEEEE HIEEEEE HIEEEEE HIEEEEE HIEEEEE HIEEEEE	: LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI	: HLLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEELHH HLLLLEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH	IHHL IHHL IHHL IHHL IHHL IHHL IHHL IHLL IHLL IHLL
A_Avr I_Avr J_Vir M_Avr Z_Avr Y_Avr O_Avr Q_Avr X_Vir E_Avr R_Avr	: LEEEEEELLLEE LEEEEEEELLLEE LEEEEEELLLEE LEEEEEE	: EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI	: IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE IEEEEEEELLE	: EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH	I HIEEEE HIEEEE HIEEEE HIEEEE HIEEEE HIEEEE HIEEEE HIEEEE HIEEEE	: LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLHHI LLLLLHHI LLLLLLHHI LLLLLLHHI LLLLLLHHI	: HLLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH	IHHL IHHL IHHL IHHL IHHL IHHL IHHL IHLL IHLL IHHL
A_Avr I_Avr J_Vir M_Avr Z_Avr Y_Avr O_Avr Q_Avr X_Vir E_Avr R_Avr L_Avr	: LEEEEEELLLEE LEEEEEELLLEE LEEEEEELLLEE LEEEEEE	: EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI	: IEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE	: EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH	HIEEEE HIEEEE HIEEEE HIEEEE HIEEEE HIEEEE HIEEEE HIEEEE HIEEEE	: LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI	: HLLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH	IHHL IHHL IHHL IHHL IHHL IHHL IHHL IHHL
A_Avr I_Avr J_Vir M_Avr Z_Avr Y_Avr O_Avr Q_Avr X_Vir E_Avr R_Avr L_Avr S_Avr	: LE EEEEEELLLEE LE EEEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE LE EEEEEELLLEE	: EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI	: IEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE	: EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH		: LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI	: HLLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH	HHL HHL HHL HHL HHL HHL HHL HHL HHL HHL
A_Avr I_Avr J_Vir M_Avr Z_Avr Y_Avr O_Avr Q_Avr X_Vir E_Avr R_Avr L_Avr S_Avr T_Vir	: LE EEEEEELLLEE LE EEEEEELLLEE	: EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI	: IEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE	: EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH		: LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI	: HLLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH	HHL HHL HHL HHL HHL HHL HHL HHL HHL HHL
A_Avr I_Avr J_Vir M_Avr Z_Avr Y_Avr O_Avr Q_Avr X_Vir E_Avr K_Avr E_Avr R_Avr L_Avr S_Avr T_Vir G_Vir	: LE EEEEELLLLEE LE EEEEELLLEE LE EEEEELLLEE	: EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI EEEEELI	: IEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEEE	: EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH EEEEELLLLHH		: LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLHHI LLLLLLHHI	: HLLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH HLLLLEEEEELHH	HHHL HHHL HHHL HHHL HHL HHL HHL HHL HHL

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	А	2	2	-	2	75	75
AvrRvi6_B04_A_noSP.pdb	А	0.52	0.98	96%	75	75	75



• 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





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



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

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A_Avr	YSCCFEVLGORDVATO	GVFANGGVFTWAPRTDC	IIEIN <mark>T</mark> NAESC <mark>S</mark> GWRWRY	LSGTSCKSLGLPLAYLGT	APRSOCN
I_Avr	YSCCFEVLGQRDVAT	GVFANGGVFTWAPRTDC	IIEIN <mark>T</mark> NAESCPGWRWRY	LSGTSCKSLGLPLAYLGT	APRSQCN
J_Vir	YSCCFEVLGQKDVAT	GVFANGGVFTWAPRTDC	IIEIN <mark>T</mark> NAESC <mark>S</mark> GWRWRY	LSGTSCK <mark>T</mark> LGLPLAYLGT	APRSQCN
M_Avr	YSCCFEVLGORDVATO	GVFANGGVFTWAPRTDC:	IIEINANAESC <mark>S</mark> GWRWRY	LSGTSCKSLGLPLAYLGT?	APRSQCN
Z_Avr	YSCCFEVLGQKDVAT	GVFANGGVFTWAPRTDC:	IIEIN <mark>T</mark> NAESCPGWRWRY	L <mark>P</mark> GTSCKSLGLPLAYLGT	APRSQCN
Y_Avr	YSCCFEVLGQKDVATO	GVFANGGVFTWAPRTDC:	IIEINANAESC <mark>S</mark> GWRWRY	LSGTSCKSLGLPLAYLGT	APRSQCN
O_Avr	YSCCFEVLGQKDVAT	GVFANGGVFTWAPRTDC:	IIEINANAESCPGWRWRY	LSGTSCKSLGLPLAYLGT	APRSQCN
Q_Avr	YSCCFEVLGQKDVAT	GVFANGGVFTWAPRTDC:	IIEINANAESCPGWRWRY	L <mark>P</mark> GTSCKSLGLPLAYLGT	APRSQCN
X_Vir	YSSCFEVLGQKDVAT	GVFANGGVFTWAPRTDC:	IIEIN <mark>T</mark> NAESCPGW <mark>RW</mark> RY	LSGTSCK <mark>T</mark> LGLPLAYLGT	APRSQCN
E_Avr	YSCCFEVLGQKDVAT	GVFANGGVFTWAPRTDC	IIEIN <mark>T</mark> NAESCPGW <mark>RW</mark> RY	LSGTSCK <mark>T</mark> LGLPLAYLGTA	APRSQCN
P_Vir	YSCCFEVLGQKDVAT	GVFANGGVFTWAPRTDC.	IIEINANAESCPGW <mark>RW</mark> RY	LSGTSCK <mark>T</mark> LGLPLAYLGT <i>I</i>	APRSQCN
R_Avr	YSCCFEVLGQKDVAT	GVFANGGVFTWAP <mark>S</mark> TDC:	IIEINANAESCPGW <mark>RW</mark> RY	L <mark>P</mark> GTSCKSLGLPLAYLGT	APRSQCN
L_Avr	YSCCFEVLGQK <mark>H</mark> VATO	GVFANGGVFTWAPRTDC.	IIEINANAESCPGW <mark>RW</mark> RY	LSGTSCK <mark>T</mark> LGLPLAYLGTA	APRSQCN
S_Avr	YSCCFEVLGQKDVAT	GVFANGGVFTWAP <mark>S</mark> TDC:	IIEINANAESC <mark>S</mark> GW <mark>RW</mark> RY	LSGTSCK <mark>T</mark> LGLPLAYLGT#	APRSQCN
T_Vir	YSCCFEVLGQKDVAT	GVFANGGVFTWAP <mark>L</mark> TDC:	IIEINANAESC <mark>S</mark> GW <mark>RW</mark> RY	LSGTSCK <mark>T</mark> LGLPLAYLGTA	APRSQCN
G_Vir	YSCCFEVLGQKDVAT	GVFANGCVFTWAPRTD <mark>R</mark>	IIEIN <mark>T</mark> NAESC <mark>S</mark> GWRWRY	LSGTSCK <mark>T</mark> LGLPLAYLGT#	APRSQCN
H_Vir	YSCCFEVLGQKDVAT	GV <mark>S</mark> ANGCVFTWAPRTDC:	IIEIN <mark>T</mark> NAESC <mark>S</mark> GW <mark>R</mark> WRY	LSGTSCK <mark>T</mark> LGLPLAYLGT#	APRSQCN
	·	: :	:	: :	
A_Avr	LEEEEEEELLLLEEEE	EEEELLIEEEEEEELLEI	EEEEELLLLLHHHL <mark>EE</mark> EE	LLLLLLHHHLLLLLEEEEH	ELHHHHL
I_Avr	LEEEEEEELLLLEEEE	EEEELLI <mark>E</mark> EEEEEELLEI	EEEEELLLLLHHHL <mark>EE</mark> EE	LLLLLLHHHLLLLLEEEEH	ELHHHHL
J_Vir	LEEEEEEELLLLEEEE	EEEELLIEEEEEELLEI	EEEEELLLLHHHL <mark>EE</mark> EE	LLLLLLHHHLLLLLEEEEH	ELHHHHL
M_Avr	LEEEEEEELLLLEEEE	EEEELLIEEEEEELLEI	EEEEELLLLHHHL <mark>EE</mark> EE	LLLLLHHHLLLLLEEEEH	ELHHHHL
Z_Avr	LEEEEEEELLLLEEEE	EEEELLIEEEEEEELLEI	EEEEELLLLHHHL <mark>EE</mark> EE	LLLLLLHHHLLLLLEEEEH	ELHHHHL
Y_Avr	LEEEEEEELLLLEEEE	EEEELLIEEEEEEELLE	EEEEELLLLHHHLEEEE	LLLLLLHHHLLLLLEEEEH	ELHHHHL
O_Avr	LEEEEEEELLLLEEEE	EEEELLIEEEEEELLEI	EEEEELLLLHHHLEEEE	LLLLLLHHHLLLLLEEEEH	ELHHHHL
Q_Avr	LEEEEEEELLLLEEEE	EEEELLIEEEEEELLE	SEEEELLLLLHHHLEEEE	LLLLLHHHHLLLLEEEEH	ELHHHHL
X_Vir	LECEEEEEELLLLEEEE	EEEELLIEEEEEEELLE)	SEEEELLLLLHHHLEEEE	LLLLLLHHHHLLLLLEEEEH	ELHHHLL
E_Avr	LECEEEEEELLLLEEEE	EEEELLIEEEEEELLE	SEEEELLLLLHHHLEEEE	LLLLLLHHHLLLLLEEEEH	ELHHHHL
P_Vir	LECEEEEEELLLLEEEE	EEEELLIEEEEEELLE	SEEEELLLLLHHHLEEEE	LLLLLLHHHHLLLLLEEEEF	ELHHHLL
R_Avr	LEFEFEFEETTTTFFEE	SEEELLIEEEEEEELLEI	SEEEELLLLLHHHLEEEE	LTTTTHHHHTTTTEEEEE	ELHHHHL
L_Avr	LECEEEEEELLLLEEEE	EEEELLIEEEEEELLEI	SEEEELLLLLHHHLESEE	LLLLLLHHHHLLLLLEEEEE	ELHHHHL
S_Avr	LEEEEEEEELLLLEEEE	SEERLLIEEEEEEELLEI	SEEEELLLLLHHHLEEEE	LLLLLHHHHLLLLEEEE	стнннг
T_Vir	LEEEEEEEELLLLEEEE	SEERTTIEEEEEETTE!	SEEEELLLLLHHHLEEEE	LLLLLHHHHLLLLLEEEE	ELHHHHL
G_Vir	LEEEEEEELLLLEEEE	EEEELLIEEEEEELLE	EEEEELLLLHHHIEEEE	LLLLLL <mark>LLL</mark> LLLLEEEEH	SLHHHLL
H_Vir	TERRERERETTTTEEEE	SEERTTEEEEEEETTE!	REFERENTITITHHHT <mark>E</mark> EEE	нтттттнинтттттеееен	ытнитр

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

Infiltration			Resulti	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	ſ		
T-	Cf4_N	AvrRvi6 B04			On all		
T-	Rvi6	avrRvi6_1180			On all		
			nrc4	nrc2/3			
R/Avr	Rvi6	AvrRvi6 B04	WT NRC	nrc2/3/4			
			WT Angers				
T+nrc2/3	Pto	AvrPto	WT Angers		nrc2/3		
T+ nrc2/3/4	Rx	СР	WT Angers		nrc2/3/4		
Tunnal	Daiblb2	Aurohik 2	WT Angers		Ĩ.		
I + MFC4	Rpiblb2	AVIDIDZ	nrc4				

Transient expression in N. benthamiana



nrc2/3

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

Infiltration			Resulti	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	
			nrc4	nrc2/3		
R/Avr	Rvi6	AvrRvi6 B04	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	
Ti nrol	Daiblb2	Aurohik 2	WT Angers			
I+ nrc4	Rpiblbz	AVIDIDZ	nrc4			

Transient expression in N. benthamiana



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

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

	Infiltratio	on	Resulti	Resulting reaction		
1			HR	Very weak HR	No HR	
			bak1		sobir1	
T+	Cf4_A	Avr4_A	WT SOBIR1			
		<u> </u>	WT Angers			
			bak1	sobir1		
T+	Cf4_N	Avr4_N	WT SOBIR1			
			WT Angers			
T-	Cf4_N	AvrRvi6 B04			On all	
T-	Rvi6	avrRvi6_1180			On all	
D/Aur	Duil		WT SOBIR1	bak1	sobir1	
R/AVI	RVI0	AVI KVI6 BU4	WT Angers			

Transient expression in N. benthamiana



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

Infiltration			Resulting reaction		
1			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



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