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The type III effector RipAX2 confers avirulence of *Ralstonia solanacearum* to the eggplant AG91-25, carrying the resistance gene Ers1

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- Among Solanaceae, highest bacterial wilt-resistance levels have been observed in eggplant.
- The resistance of AG91-25 is conferred by a combination of the major locus Ers1 and QTLs (talk S. Salgon, session 4).
- To decipher the molecular basis governing *R.solanacearum*-eggplant interactions, we investigated the contribution of type III effectors to the avirulence to AG91-25.
- We present the first results on the avirulence function of RipAX2, a Zn-dependant protease.

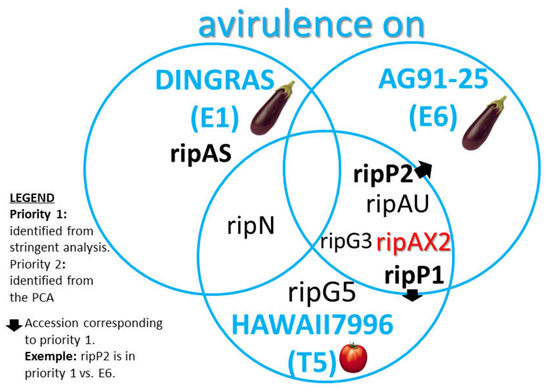
The approach

Association genetics, PENSEC *et al*, 2015. (http://dx.doi.org/10.1094/PHYTO-06-15-0140-R)

Towards the Identification of Type III Effectors Associated with *Ralstonia solanacearum* Virulence on Tomato and Eggplant

Flora Pensec, Aurore Lebeau, M. C. Daunay, Frédéric Chiroleu, Alice Guidot, and Emmanuel Wicker

Type III effectors associated with avirulence on



Does the inactivation of the effector make the strain virulent on AG91-25 ?

Inoculation of GMI1000 singleT3E mutants on AG91-25 (E6, resistant) and MM738 (E8, susceptible) in a soil-soak experiment mimicking the natural infection conditions

Does the injection of the effector induce a plant response ?

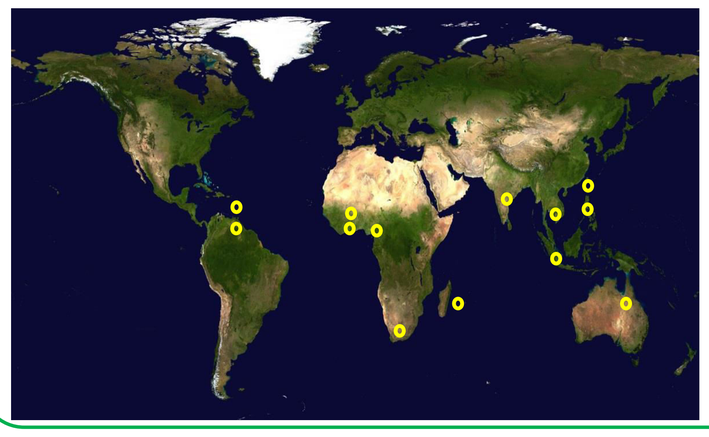
- HR in the leaf ?

Agrobacterium tumefaciens (At) mediated injection (GMI1000 allele) in the leaves of E6 and E8.

- Defences impairing the internal bacterial growth in leaves and stem ?

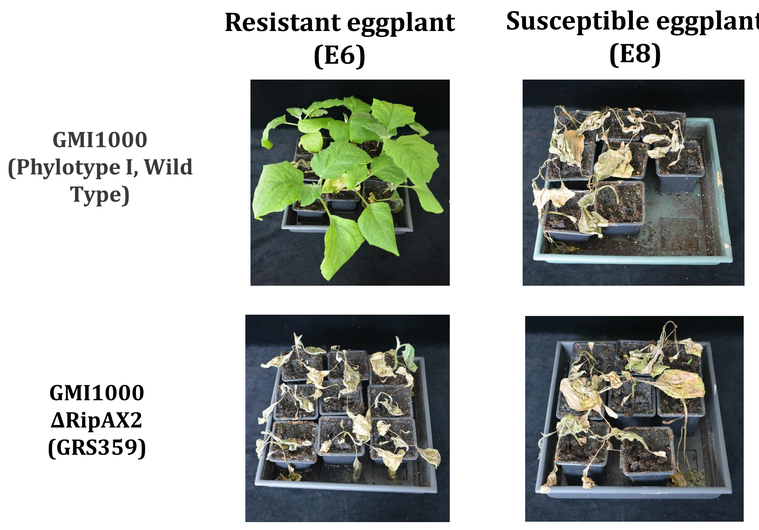
Heterologous expression in *Pseudomonas syringae* DC3000 (Pst) and *P. fluorescens* (EtHAN)

What is the effector prevalence and allelic diversity in natural pathogen populations ? Is avirulence conferred by specific alleles ?



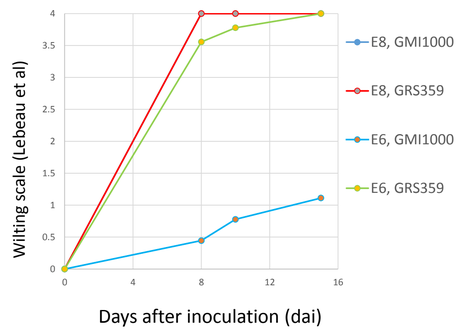
- 91 strains from 13 geographical locations
  - phylotype I (66) , IIA (9), IIB (10), III (6)
- Virulence phenotyping on E6 and E8 by soil-soak experiment (28 days, 30°C day /24°C night): 69 strains
- Full-length PCR and sequencing, including the upstream promoter region
- 9 complete genomic sequences, phylotype I and III (Guinard et al 2016, Genome Announcements; doi: 10.1128/genomeA.01415-15)

1. RipAX2 is strongly involved in the control of GMI1000 by AG91-25



- 15 effector mutants were tested.
- The ΔRipAX2 mutant was virulent to AG91-25.
- RipAX2 induces HR on *Solanum torvum*, a wild relative of eggplant (Nahar et al, 2014)

Wilting symptoms over time



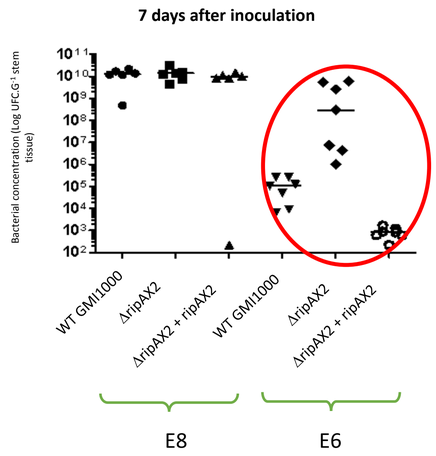
Each treatment consisted of 8 plants (10 mL * 10^8CFU/mL), repeated 3 to 4 times.

2. RipAX2 does not induce HR in leaves, and may be recognized in the stem

Leaf infiltration of RipAX2-expressing At and Pst does not trigger E6 resistance

RipAX2-carrying At injections in E6 leaves, 120 h post-inoculation

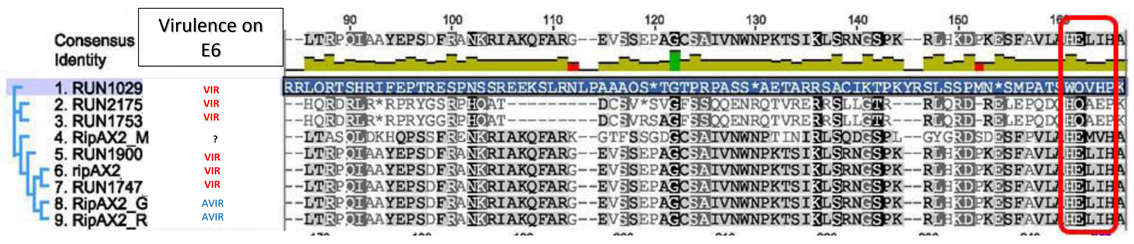
In E6 stem, RipAX2 induces a 4 magnitude-decrease of the bacterial load.



An organ-specific control of GMI1000 by AG91-25 ?

3. RipAX2-eggplant E6 : a different story than with *Solanum torvum*

- The critical residue for avirulence to *S. torvum* is E_{149}, within the putative Zinc-binding motif HExxH).



- In E6 -avirulent (GMI1000, RS1000) AND virulent strains, E_{149} and the HELIH motif are conserved.

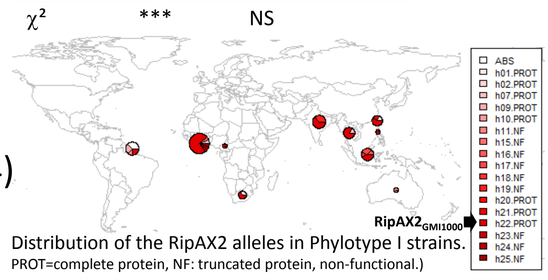
4. RipAX2 is highly prevalent in Rs natural populations

- Significantly more present in phylotype I, preferentially absent in phylotype II.

Phylotype	Abs	Pres	Total	χ^2
I	4	62	66	***
IIA	5	4	9	**
IIB	7	3	10	***
III	2	4	6	NS
Total	18	73	91	
χ^2	***	NS		

26 alleles

- **One dominant allele: $RipAX2_{GMI1000}$ (60.3%)**
  - present in I, IIA, IIB
- Allelic richness: Asia (8) > Africa (7) > South America (4) > Indian Ocean (1)
- 13 truncated proteins



Distribution of the RipAX2 alleles in Phylotype I strains.
PROT=complete protein, NF: truncated protein, non-functional.)

5. Alleles vs virulence: an ongoing study

No alleles	Protein	Phenotype on E6 (No strains) Avirulent	Virulent
1	ABSENTE	7	10
13	Functional	31	8
13	Truncated	9	4
Total		47	22

- 3 functional alleles associated with virulence

- Strains carrying the GMI1000 allele are not all avirulent

Phenotype on E6 (No strains) Avirulent	Virulent
25	5

We identified three potential strategies for Rs to bypass E6 resistance: (i) gene deletion, (ii) pseudogenisation, (iii) modification of the promoting regions. Deeper functional analyses are needed.

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