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

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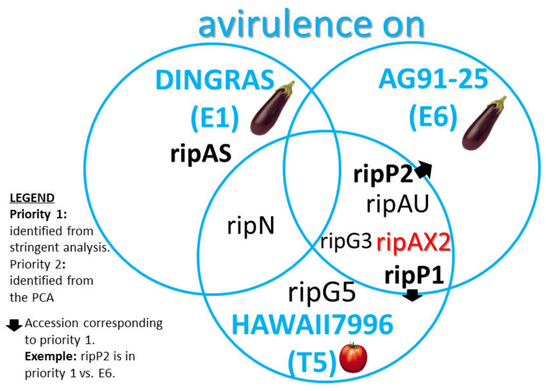
Type III effectors associated with avirulence on



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 ?

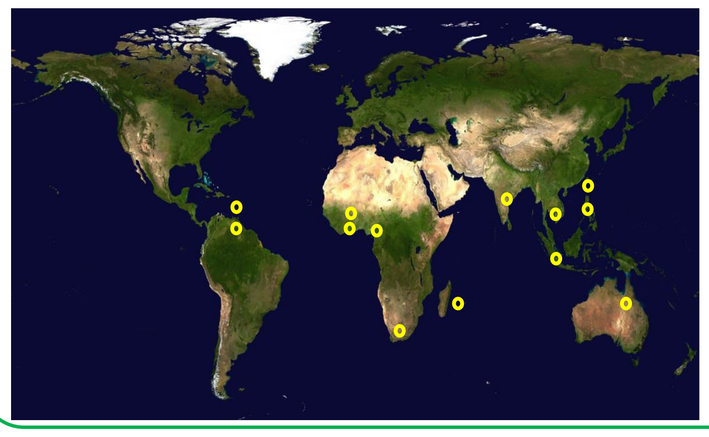
- HR in the leaf ?

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

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

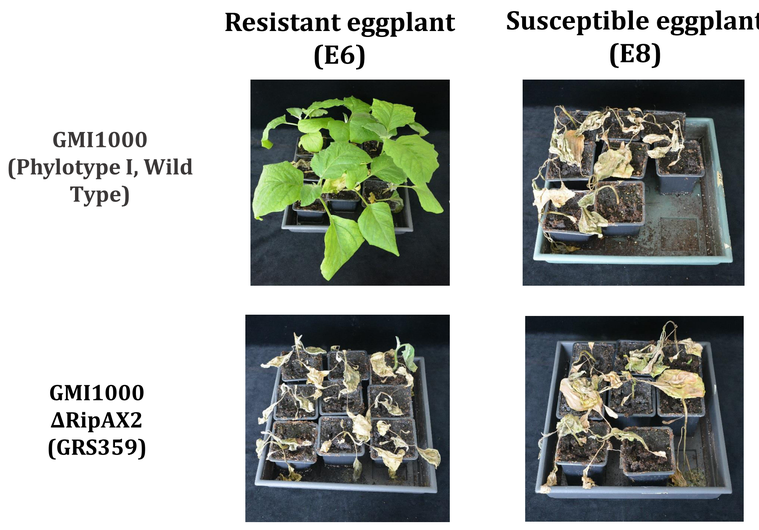
Heterologous expression in *Pseudomonas syringae* DC3000 (Pst) and *P. fluorescens* (EtHAN)

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



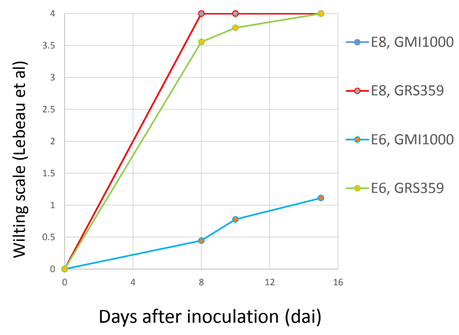
- 91 strains from 13 geographical locations
  - phylotype I (66) , IIA (9), IIB (10), III (6)
- Virulence phenotyping on E6 and E8 by soil-soak experiment (28 days, 30°C day /24°C night): 69 strains
- Full-length PCR and sequencing, including the upstream promoter region
- 9 complete genomic sequences,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



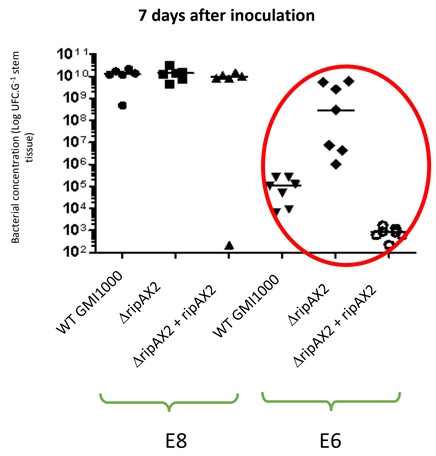
Each treatment consisted of 8 plants (10 mL * 10^8CFU/mL), repeated 3 to 4 times.

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

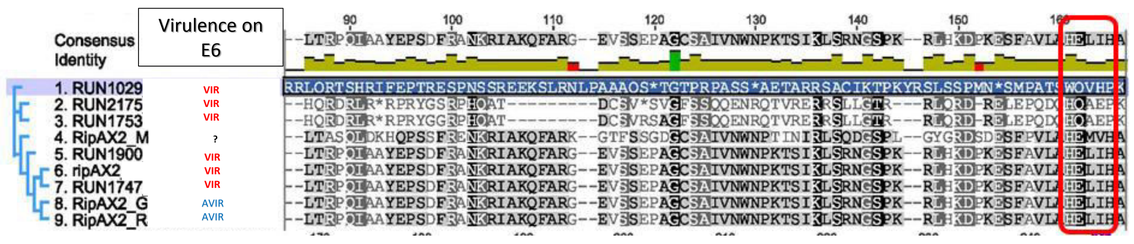
In E6 stem, RipAX2 induces a 4 magnitude-decrease of the bacterial load.



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.

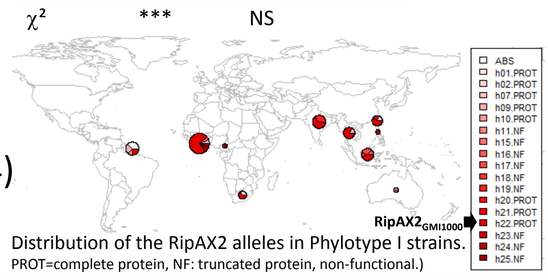
4. RipAX2 is highly prevalent in Rs natural populations

- 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.

Acknowledgements

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