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

- 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, PENGEC et al, 2015.**

![_diagram]

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

- Inoculation of GM1000 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?

**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)

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

- The critical residue for avirulence to S. torvum is E249G within the putative Zinc-binding motif HEXXH).
- In E6 -avirulent (GM1000, Rs1000) AND virulent strains, E149 and the HELIHM motif are conserved.

**4. RipAX2 is highly prevalent in Rs natural populations**

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

**26 alleles**

- One dominant allele: RipAX2-GMI1000G (60.3%)
  - present in I, IIA, IIB
- Allelic richness: Asia (8) > Africa (7) > South America (4) > Indian Ocean (1)
- 13 truncated proteins

**Acknowledgements**

Sylvain LEBON, Jean-Michel BAPTISTE, Fanny MAILLOT, Edith LALLEMAND-MAMOSA, Marie TERVILLE, Frédéric CHIROLELU

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

<table>
<thead>
<tr>
<th>Phenotype on E6 (No strains)</th>
<th>Resist</th>
<th>Susc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Avirulent</strong></td>
<td><strong>Virulent</strong></td>
<td></td>
</tr>
<tr>
<td>GMI1000</td>
<td>GMI1000</td>
<td></td>
</tr>
<tr>
<td>E6</td>
<td>E8</td>
<td></td>
</tr>
</tbody>
</table>

*Resistant eggplant (E6) | Susceptible eggplant (E8)*

- Wilting symptoms over time
- Each treatment consisted of 8 plants (60 CFU/mL), repeated 3 to 6 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

An organ-specific control of GM1000 by AG91-25?

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

**3. RipAX2-carrying At injections in E6 leaves, 12th post-inoculation**

**5. Alleles vs virulence: an ongoing study**

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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- 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)

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