

Transcriptomic regulation in pepper during the interaction with *Phytophthora capsici*

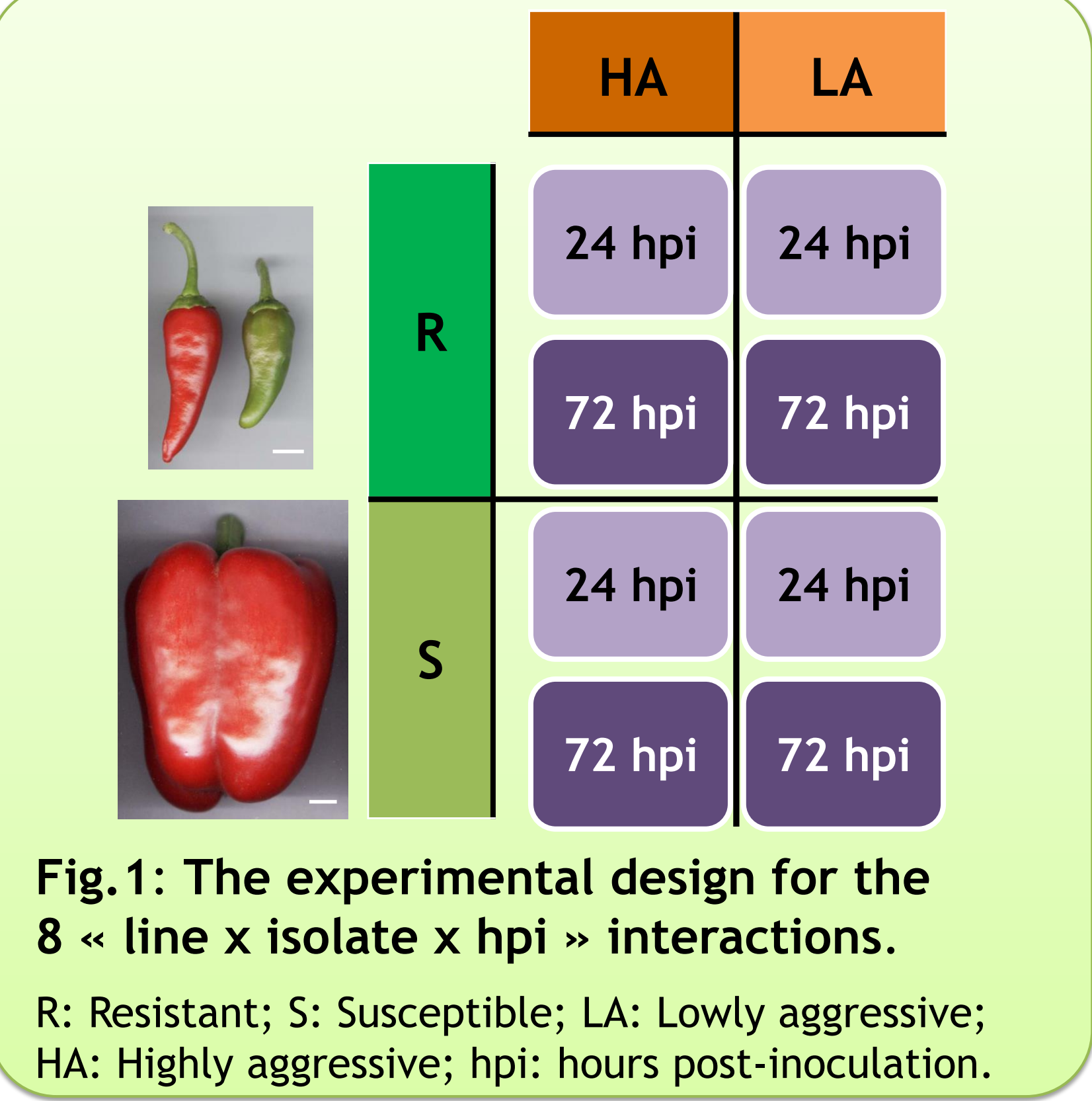


Gaëtan MAILLOT¹, Emmanuel SZADKOWSKI¹, Anne MASSIRE¹, Jean-Paul BOUCHET¹, Véronique BRUNAUD², Marie-Laure MARTIN-MAGNIETTE², Sandrine BALZERGUE², Guillem RIGAILL², Kurt LAMOUR³, Véronique LEFEBVRE¹

¹ INRA, UR1052 GAFL, Fruit and Vegetable Genetics and Breeding Research Unit, F-84143 Montfavet Cedex, France (gaetan.maillot@inra.fr)
² INRA, UMR 9213 / UMR1403 IPS2, CNRS, Universités Paris-Sud, d'Evry, de Paris-Diderot et de Sorbonne Paris-Cité, F-91405 Orsay, France
³ Department of Entomology and Plant Pathology, the University of Tennessee, Knoxville, TN, USA

The oomycete *Phytophthora capsici* Leonian causes severe damage to peppers.

- The few pepper accessions resistant to *P. capsici* reported up to now display polygenic determinisms hindering breeding.
- To identify genes responsible for resistance, we investigated the gene expression in the pepper - *P. capsici* interaction.
- To gain resolution, we used a multifactorial approach with several pepper lines, *P. capsici* isolates and time-points of tissue sampling.



A multifactorial RNAseq analysis

- Plant and pathogen material:
 - Two pepper lines: CM334 (R for resistant) and YW (S for susceptible),
 - Two *P. capsici* isolates: Pc273 (LA for lowly aggressive) and Pc107 (HA for highly aggressive).
- Stem-infected tissues were collected in triplicate at 24 and 72 hours post-inoculation (hpi), giving 24 samples for the 8 “line x isolate x hpi” interactions (Fig.1).
- RNA extraction, library preparation and paired-end (PE) illumina sequencing were performed.
- After quality treatments by in-house scripts, reads were mapped with Bowtie2 to a reference dataset composed of pepper¹ and *P. capsici*² sequences.
- After the read counting step by in house scripts, a differential expression (DE) analysis was performed using edgeR package in R statistical software.

1 High numbers of paired-end (PE) reads and genes were analyzed.

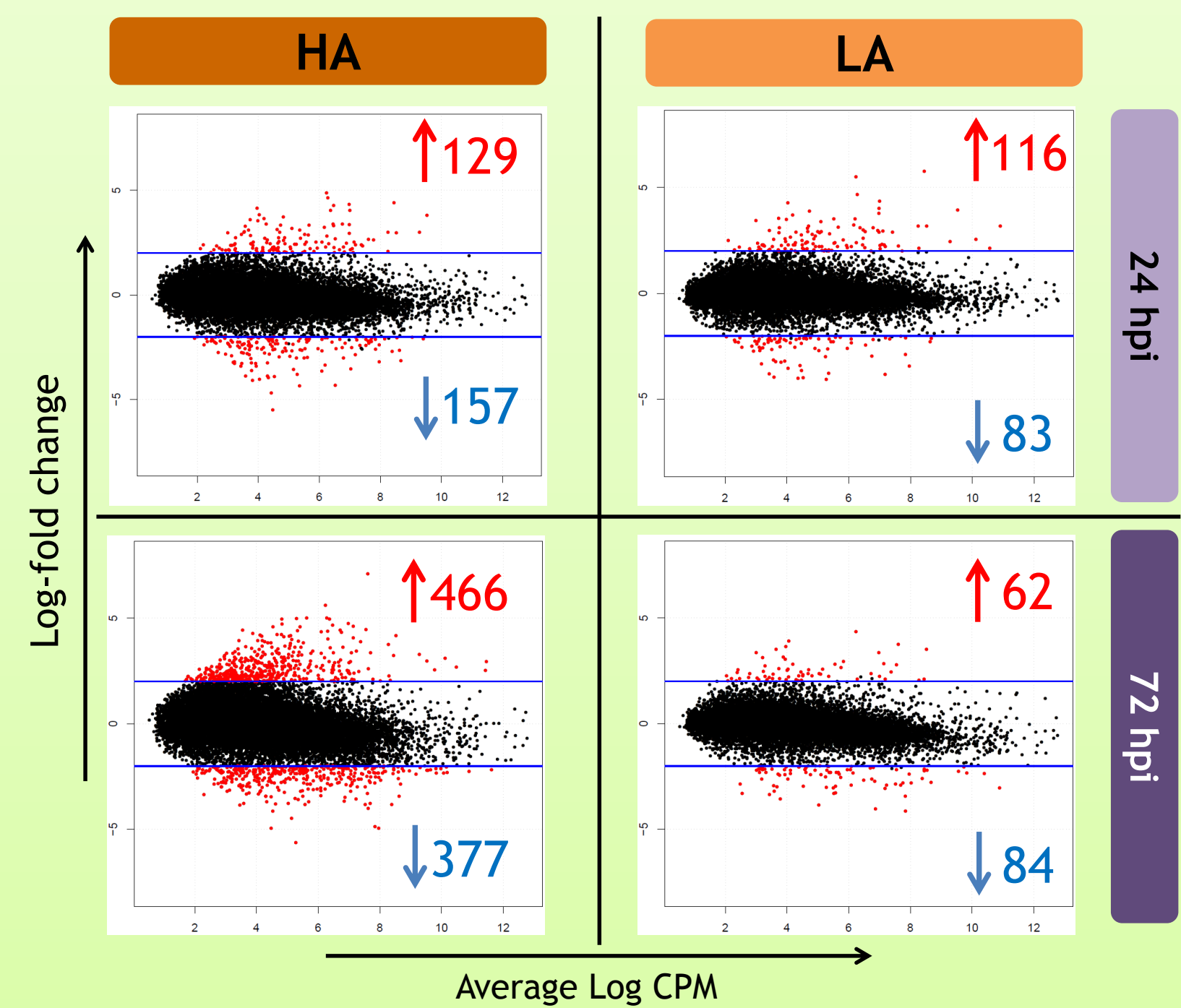
- The average number of PE reads ranged between 34,1 and 42,2x10⁶, indicating a deep sequencing (Fig.2A).
- 73 to 80% of those reads mapped to the pepper transcriptome¹ (Fig.2B).
- Based on the PE reads mapped on pepper, further analyses considered a total of 17,561 contigs equivalent to gene predictions.

A Mean library size				B Mapping efficiencies						
Pepper line	<i>P. capsici</i> isolate	Time (hpi)	Avr. PE reads (10 ⁶)	0%	20%	40%	60%	80%	100%	
R	HA	24	37,0							
		72	42,2							
	LA	24	34,9							
		72	35,6							
S	HA	24	34,1							
		72	39,3							
	LA	24	35,3							
		72	39,6							

Fig.2: Descriptive statistics and mapping efficiencies
A: Mean library size for the 8 « line x isolate x hpi » interactions of the RNAseq dataset.
B: Mean proportion of PE reads mapped to the plant (green), to *P. capsici* (orange) and unmapped (blue).

2 Do the R and S pepper lines react identically to the *P. capsici* aggressiveness and the time post-inoculation?

- The aggressive HA isolate induces more differentially expressed (DE) genes between the R and S lines than the LA isolate (Fig.3).
- DE genes increase over time for the HA isolate, but decrease for the LA isolate (Fig.3).



3 How many genes are differentially expressed (DE) between the R and S lines whatever the “isolate x hpi” interaction?

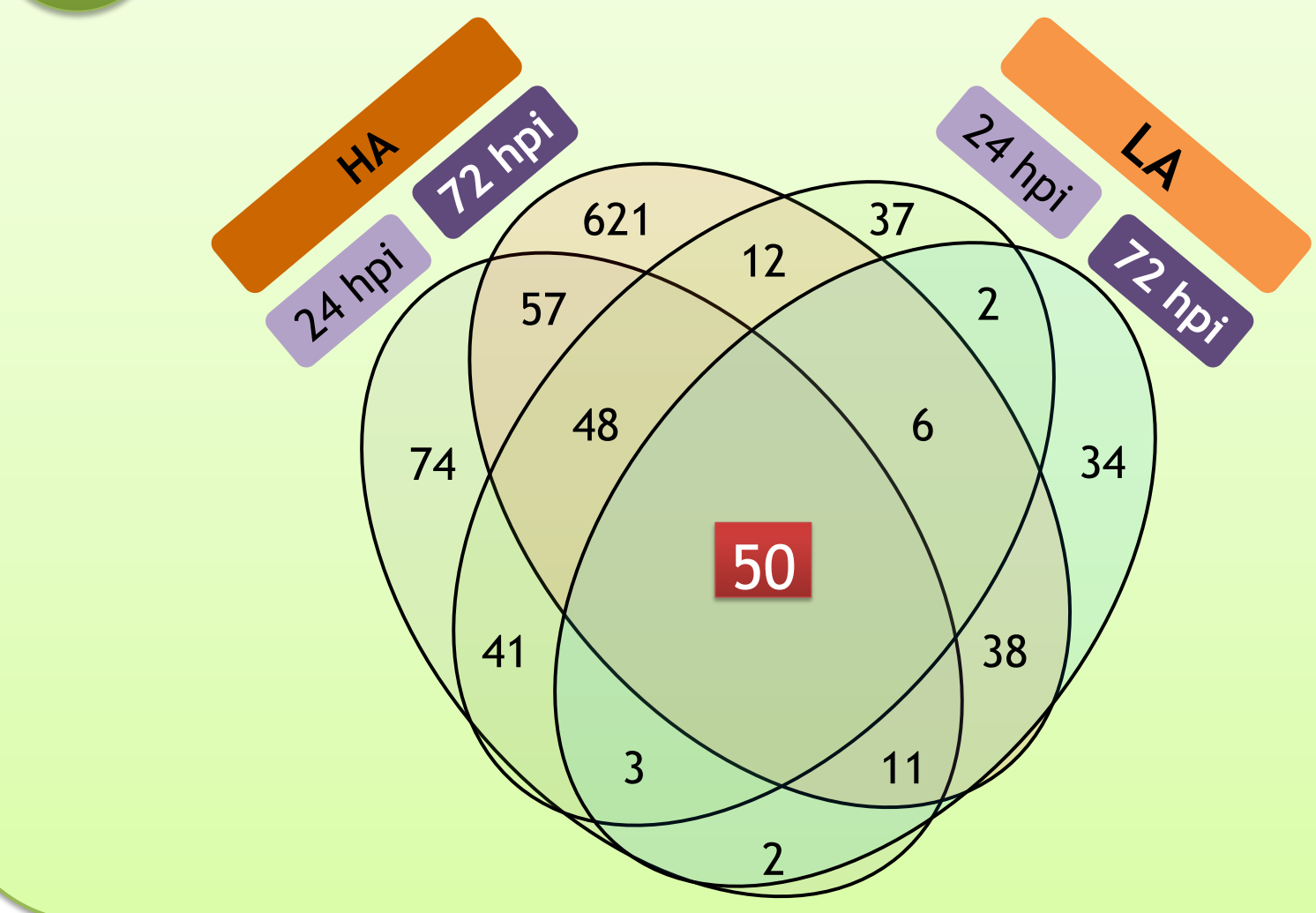


Fig.4: Venn diagram of DE genes by comparison of R and S pepper lines.
Values indicate the number of DE genes between R and S lines for the four « isolate x hpi » interactions.
HA: Highly aggressive; LA: Lowly aggressive; hpi: hours post-inoculation.

- A total of 1,086 DE genes were detected between the R and S lines, with 766 DE genes found in only one « line x isolate x hpi » interaction.
- Whatever the interaction, 50 DE genes were constantly detected (Fig.4).

Our RNAseq analysis successfully highlighted 1,086 differentially expressed genes between CM334 and YW.

- The *P. capsici* aggressiveness contrasts more the pepper gene expression than the time post-inoculation (hpi).
- A set of 50 robust pepper candidate genes were highlighted whatever the « line x isolate x hpi » interaction.
- Their role in resistance is currently analyzed with qRT-PCR expression, genome ontology, genome localization and allelic diversity.