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To cite this version:

Gaëtan Maillot, Emmanuel Szadkowski, Anne Massire, Jean-Paul Bouchet, Veronique Brunaud, et al.. Transcriptomic regulation in pepper during the interaction with Phytophthora capsici. 16. Eucarpia Capsicum and Eggplant Meeting, Sep 2016, Kecskemét, Hungary. 2016. hal-02799453

HAL Id: hal-02799453
https://hal.inrae.fr/hal-02799453
Submitted on 5 Jun 2020

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

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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.

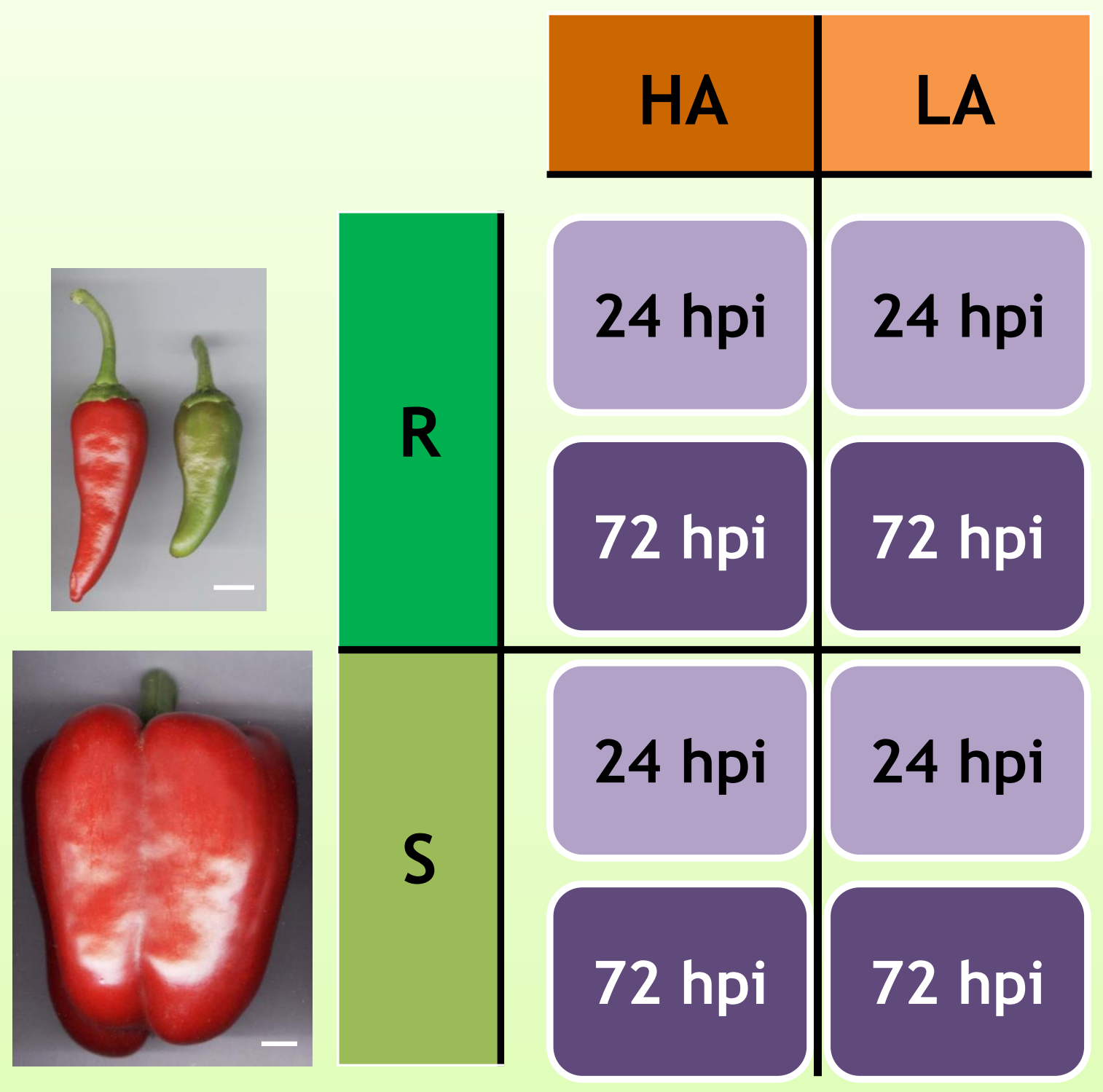


Fig.1: The experimental design for the 8 « line x isolate x hpi » interactions.

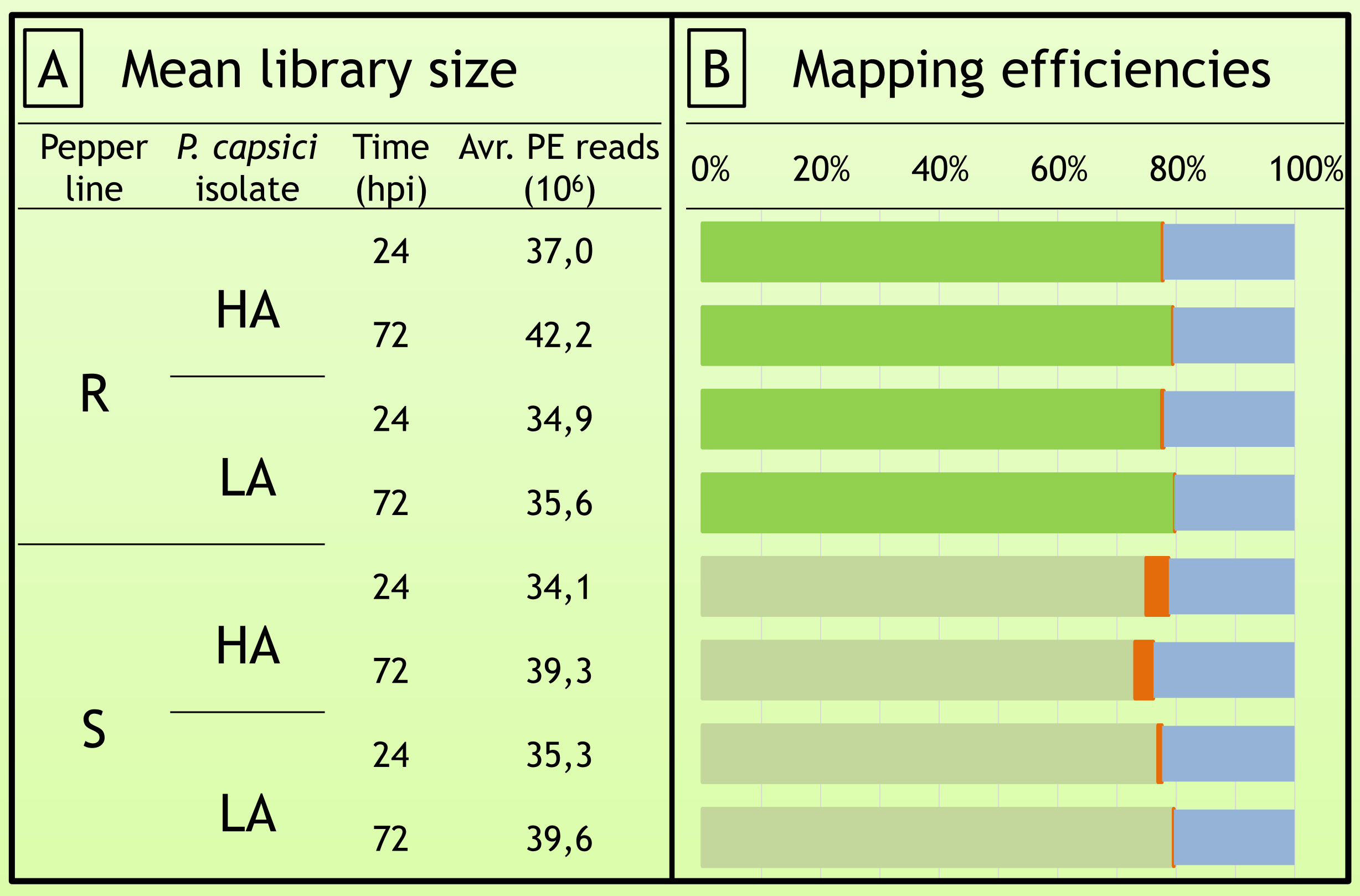
R: Resistant; S: Susceptible; LA: Lowly aggressive; HA: Highly aggressive; hpi: hours post-inoculation.

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 pepper[1] 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 42,2x10^6, indicating a deep sequencing (**Fig.2A**).
- 73 to 80% of those reads mapped to the pepper transcriptome[1] (**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

Pepper line	*P. capsici* isolate	Time (hpi)	Avr. PE reads (10^6)
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**).

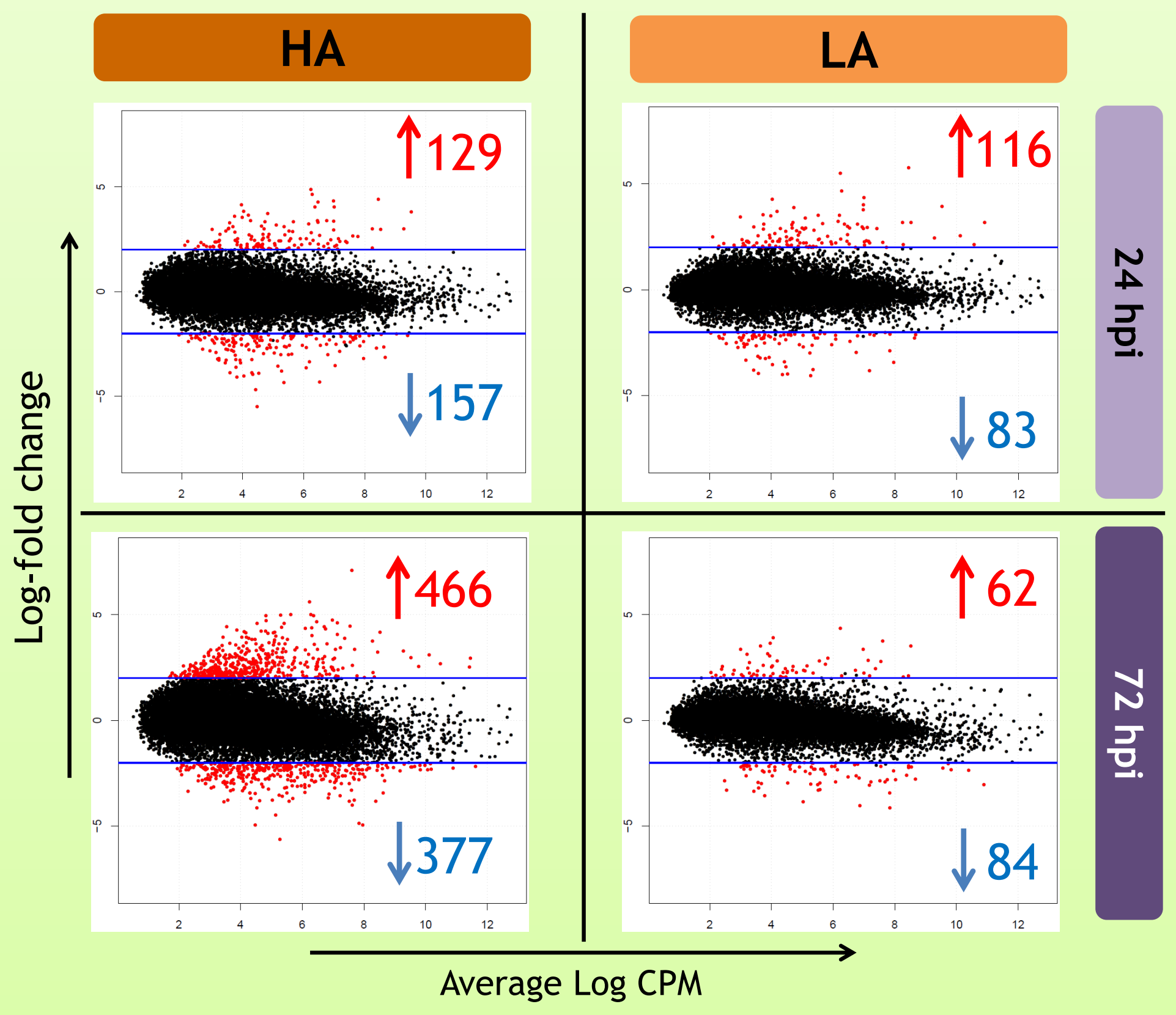


Fig.3: Differentially expressed (DE) genes between the R and S lines detected by edgeR.

Beside arrows, are indicated in red: numbers of up-regulated genes within the R line, and in blue: down-regulated genes within the R line; HA: Highly aggressive; LA: Lowly aggressive; hpi: hours post-inoculation; CPM: Count Per Million.

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

- 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**).

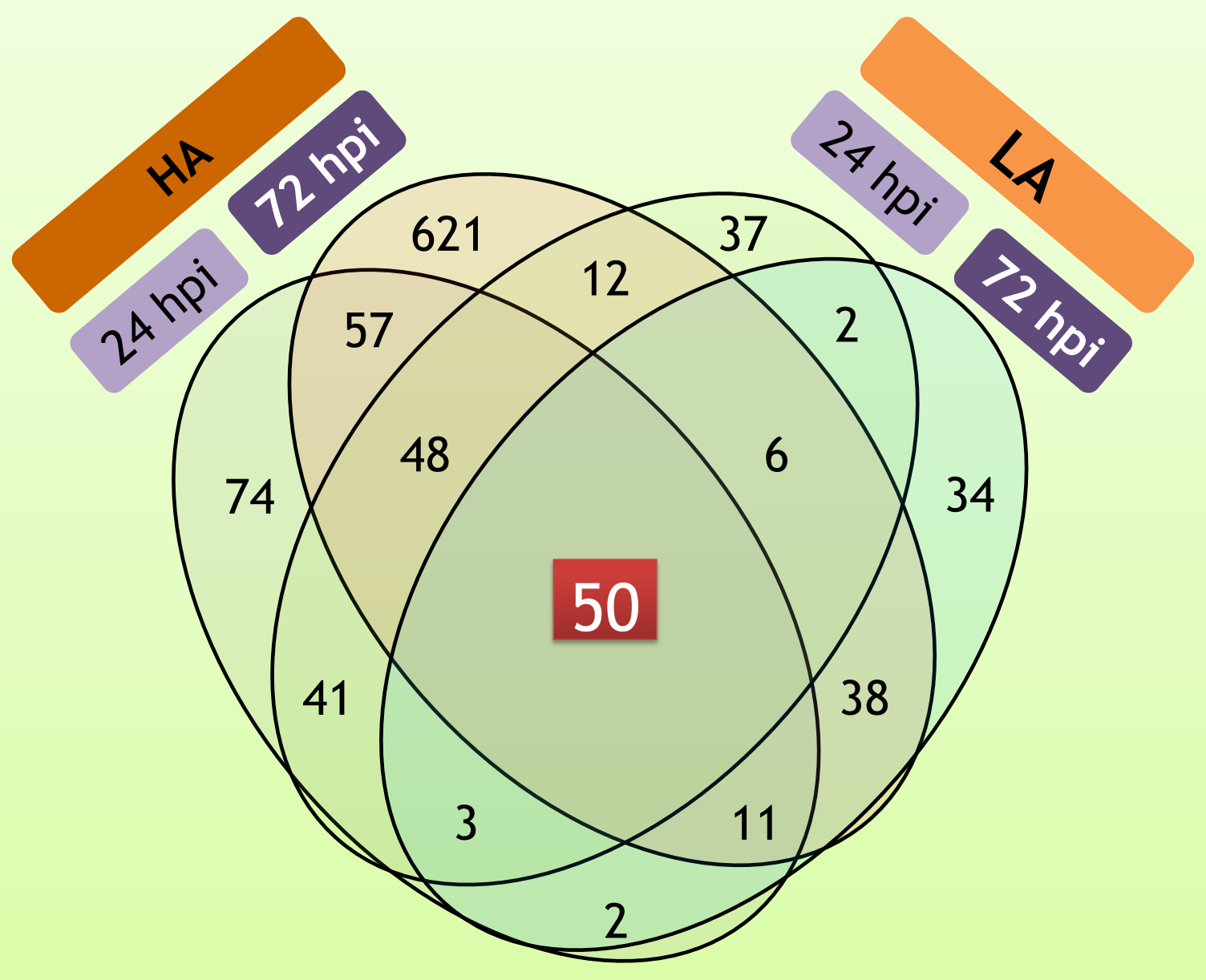


Fig.4: Venn diagram of DE genes by comparison of R and S pepper lines.

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.

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.

This project was supported by Agropolis Fondation in France under the reference ID « Protéines pathogènes 1300-002 » and by the BAP division of INRA under the reference ID « EffeCaps ».

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[1] Nicolaï *et al.*, 2012. Genetics and Molecular Research; [2] Lamour *et al.*, 2012. Molecular Plant-Microbe Interactions.

XVI th EUCARPIA Capsicum and Eggplant Meeting, September 12-14, 2016, Kecskemét, Hungary