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Regulation of effector-gene expression as concerted waves in *Leptosphaeria maculans*: a twoplayers game

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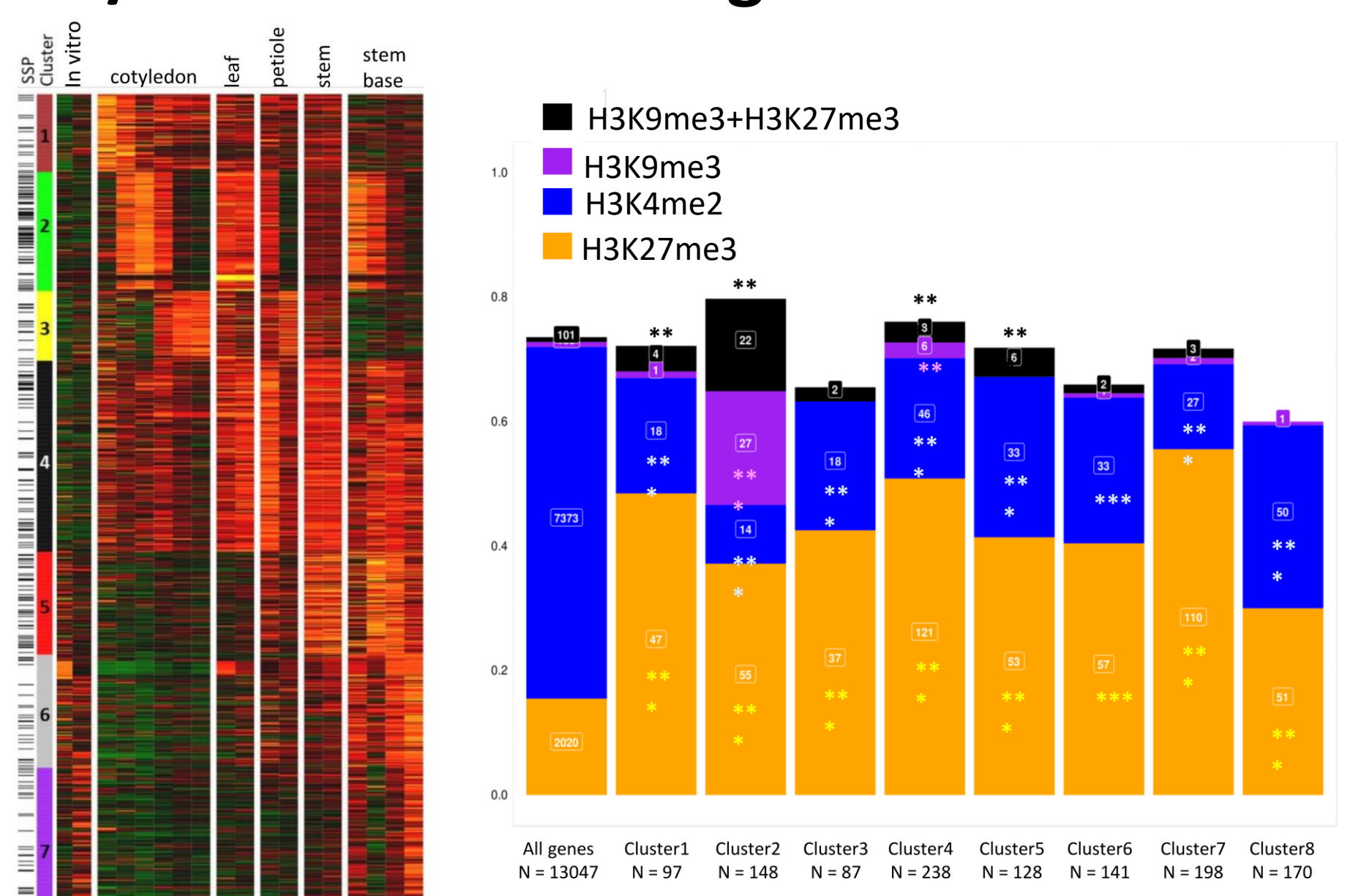
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01/ Context: effector-genes are enriched in heterochromatin domains and expressed as concerted waves during infection



- Effector-genes of *L. maculans* are expressed as waves during infection of *Brassica napus* (oilseed rape)
- Genes induced during infection are associated with heterochromatin (H3K9me3- and / or H3K27me3-domains) *in vitro*
- Previous finding that KMT1 (responsible for H3K9me3 deposition) represses effector-gene expression *in vitro*
- The transcription factor (TF) Pf2 increasingly found as involved in pathogenicity and regulation of effector-gene expression in plant-pathogenic fungi

Our working hypothesis: a two-layer regulatory network

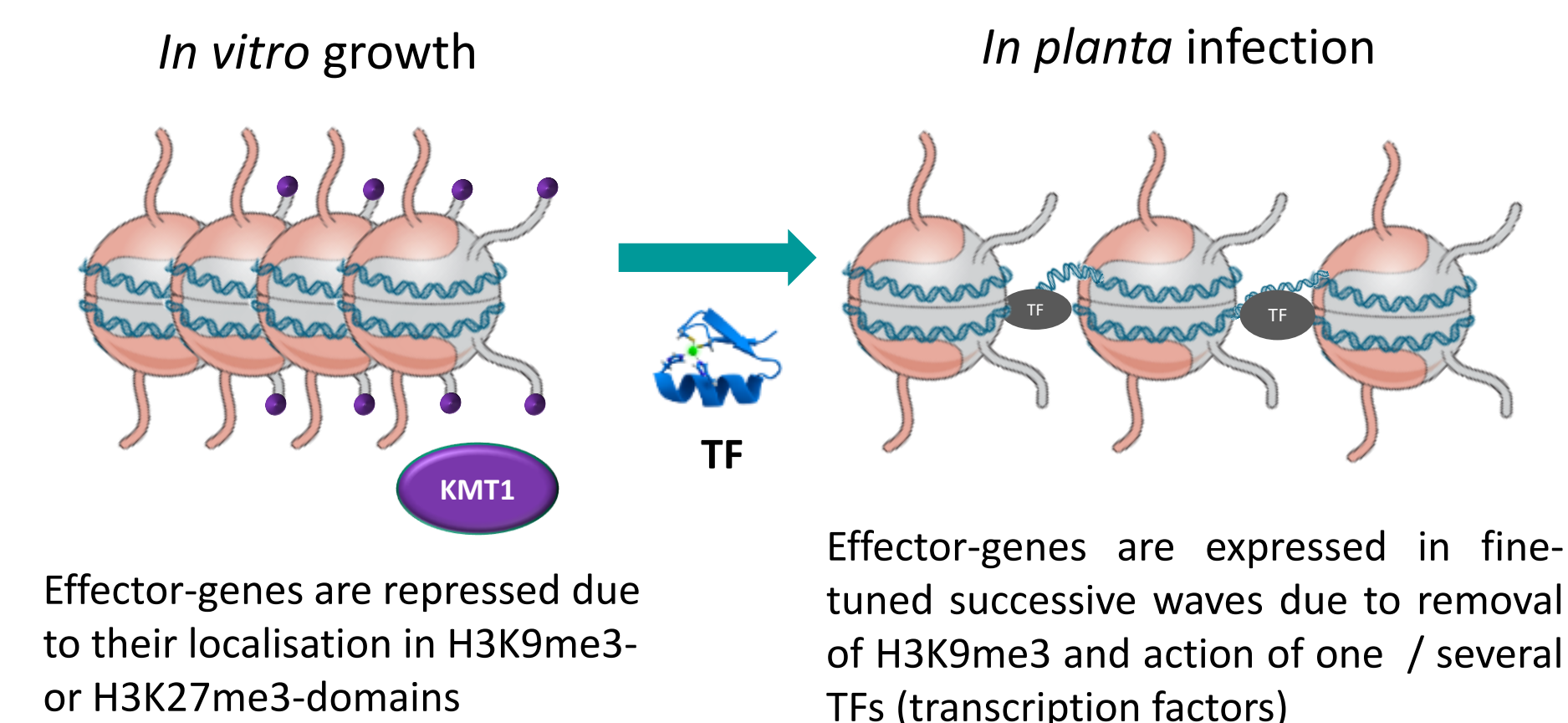


Figure adapted from Soyer et al., 2014; 2015.

Successive waves of *L. maculans* gene expression during infection of oilseed rape. Left panel: heatmap of the genes overexpressed *in planta* compared to *in vitro* conditions; right panel: up-regulated genes *in planta* are enriched in heterochromatin *in vitro*. Figure adapted from Gay et al., 2021.

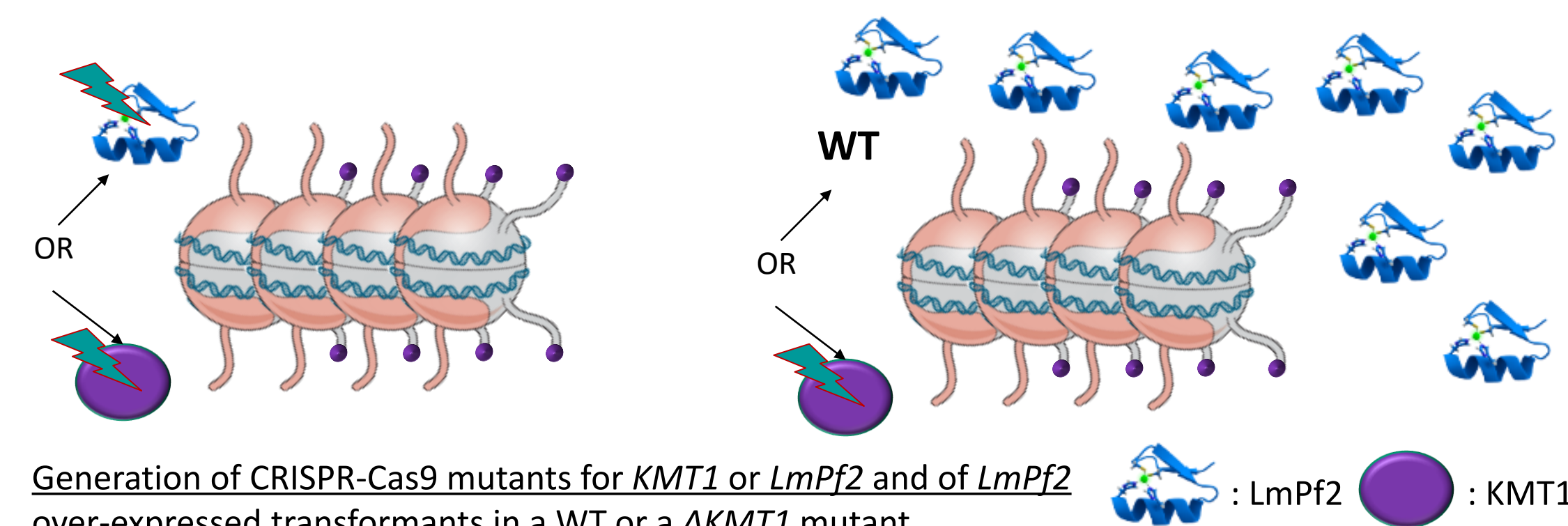
Objective: investing role of KMT1 and LmPf2 in the regulation of (effector) gene expression in *L. maculans*

02/ Methodology

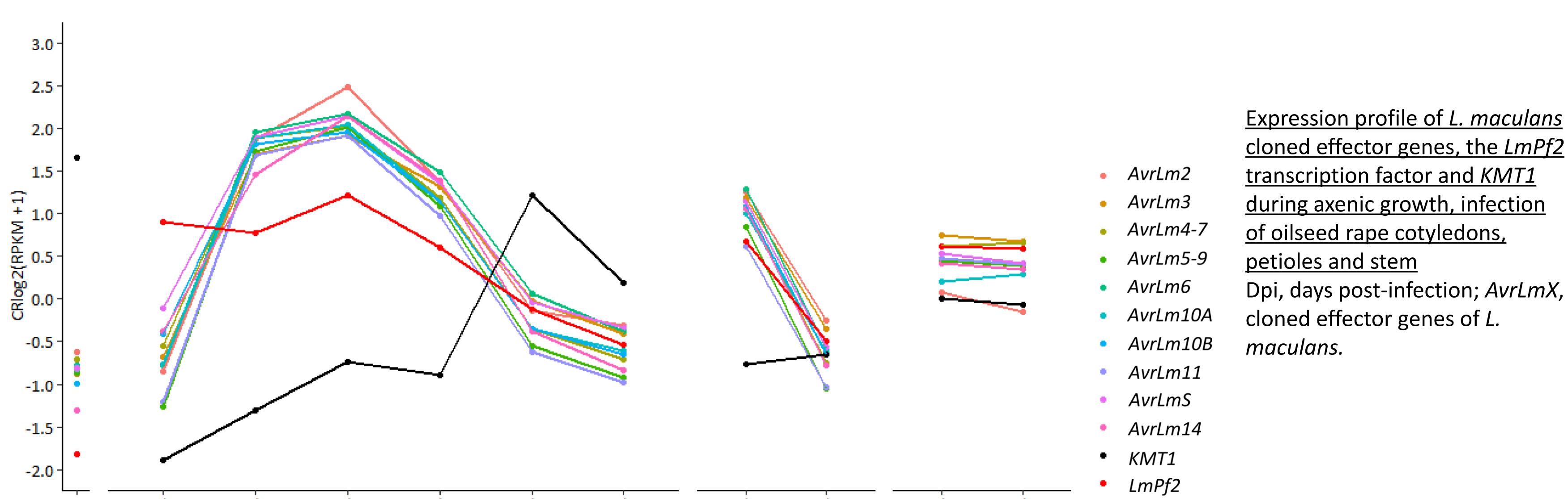
- Analysis of expression of *LmPf2*, *KMT1* and cloned effector-genes
- CRISPR-Cas9 inactivation of *LmPf2* and *KMT1*
- Over-expression of *LmPf2* in a WT and a $\Delta KMT1$ background (i.e. WT_oPf2, $\Delta KMT1$ _oPf2)
- Phenotypic characterization of the generated transformants
- Analyses of gene expression in the transformants via RNA-seq

1. Inactivations of *LmPf2* or *KMT1*

2. Over-expression of *LmPf2*



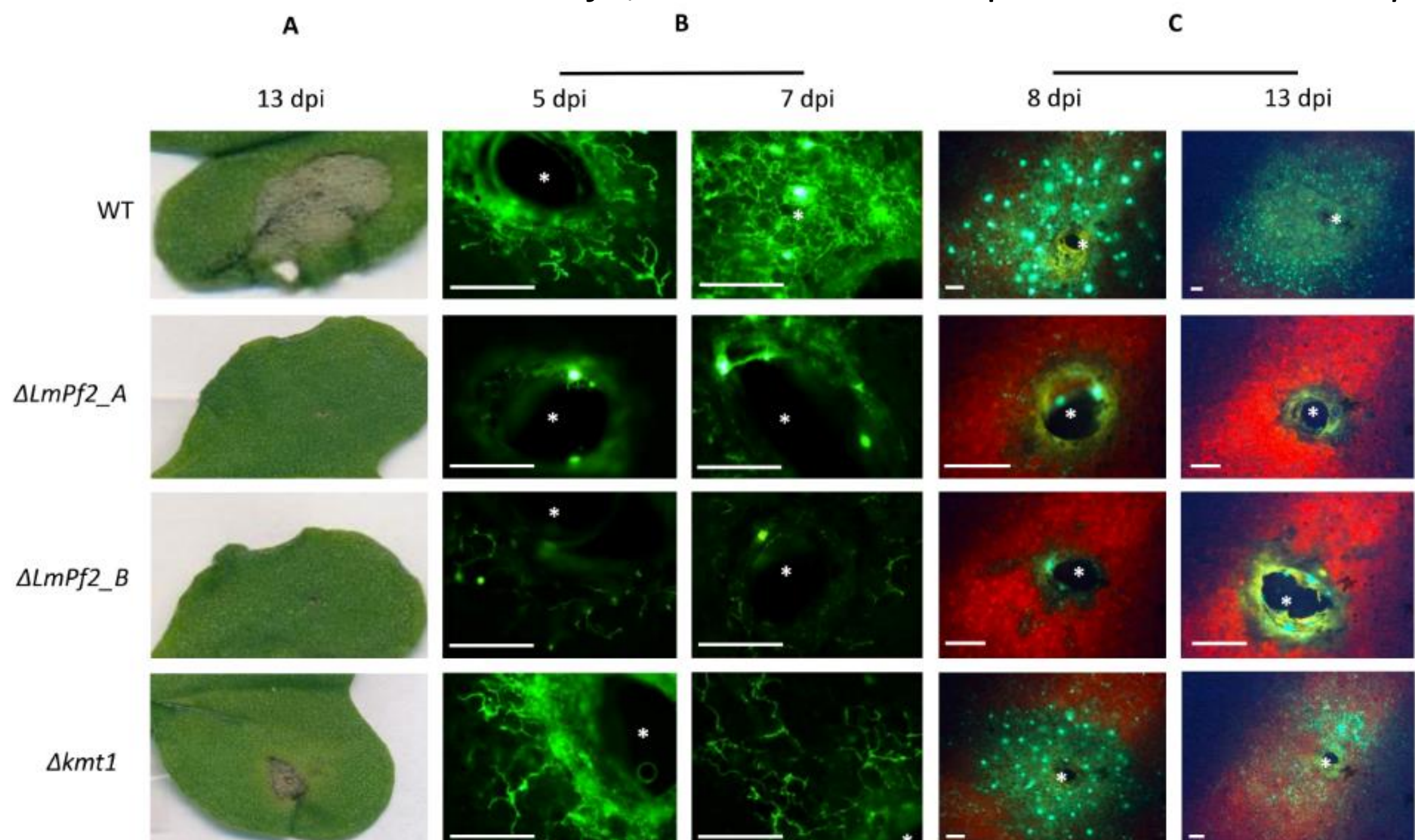
03/ Expression of *KMT1*, *LmPf2* and cloned effector-genes of *L. maculans*



- Cloned effector-genes are repressed *in vitro*, highly expressed during asymptomatic infection of oilseed rape (i.e. cotyledon 5, 7, 9 dpi; petiole 7 dpi; stem 14, 28 dpi)
- *KMT1* has an opposite expression pattern to that of effector-genes
- *LmPf2* expression follows the same pattern as cloned effector-genes

04/ *LmPf2* and *KMT1* are involved in pathogenicity and regulation of cloned effector genes of *L. maculans*

Inoculation of $\Delta LmPf2$, $\Delta KMT1$ on a susceptible cultivar of *B. napus*



Effect of *LmPf2* and *KMT1* inactivations on pathogenicity of *L. maculans*. A, Pictures of symptoms 13 dpi; B, fluorescence images of cotyledons infected by GFP mutants and the WT 5 and 7 dpi (confocal microscope); C, fluorescence images of cotyledons infected by GFP mutants and the WT 8 and 13 dpi (fluorescence binocular). White asterisk, infection site; scale bars, 1mm; dpi, days post-inoculation.

Expression of cloned effector genes *in vitro*, in the transformants generated, compared to the WT (RNA-seq data)

name	TE ^a	H3K4me2 ^b	H3K9me3 ^b	H3K27me3 ^b	$\Delta kmt1$ ^c	$\Delta LmPf2$ ^c	JN2_oPf2 ^c	$\Delta kmt1$ _oPf2 ^b
AvrLm3	yes	-	all included	-	up	-	-	up
AvrLm4-7	yes	-	all included	-	-	-	-	up
AvrLm14	yes	-	all included	-	-	-	-	up
AvrLm6	yes	-	all included	-	-	-	-	up
AvrLm2	yes	-	5'_included	-	down	-	-	up
AvrLm10_A	yes	-	all included	-	down	-	-	up
AvrLm10_B	yes	-	all included	all included	-	down	-	up
AvrLm5-Lep2	yes	-	all included	-	-	-	-	up
AvrLm5-9	yes	-	all included	-	-	-	-	-
AvrLm11	yes	-	all included	-	-	-	-	-

^aGenes located in a TE-rich genomic context;

^bGenes associated with H3K4me2, H3K9me3 or H3K27me3 during axenic culture in the wild type strain;

^cGenes deregulated in a given transformant compared to the WT strain during axenic culture.

- $\Delta LmPf2$ mutants: unable to colonize *B. napus*; $\Delta KMT1$ mutants: less symptoms than the WT
- Over-expression of *LmPf2* in a WT background: no influence on effector-gene expression
- Over-expression of *LmPf2* in a $\Delta KMT1$ background: up-regulation of effector-genes located in heterochromatin-domains

05/ *LmPf2* and *KMT1* are acting together to regulate expression of effector-genes

	Total genes	Effector genes	H3K4me2 genes ^a	H3K9me3 genes ^a	H3K27me3 genes ^a	H3K9me3/H3K27me3 genes ^a	
Up-regulated	$\Delta kmt1$	129	22*	22 [#]	3	64*	4*
	$\Delta LmPf2$	202	20	68 [#]	4	57*	5*
	WT_oPf2	247	46*	46 [#]	2	101*	6*
	$\Delta kmt1$ _oPf2_B	931	154*	233 [#]	25*	329*	24*
Down-regulated	$\Delta kmt1$	143	27*	35 [#]	10*	42*	9*
	$\Delta LmPf2$	225	40*	58 [#]	4	88*	10*
	WT_oPf2	277	19	110 [#]	1	60*	3
	$\Delta kmt1$ _oPf2_B	861	83	364 [#]	11	182*	14*

^aGenes associated with H3K4me2, H3K9me3, H3K27me3 or H3K9me3 and H3K27me3 during axenic growth of the WT isolate;

[#]genes statistically under-represented in a given category; *genes statistically enriched in a given category; Statistical analyses were performed using χ^2 , *P* value < 0.05.

Carbohydrate uptake Oxydo-reduction processes

Over-expression of *LmPf2* in a $\Delta KMT1$ background ($\Delta KMT1$ _oPf2):

- Enhanced effect on gene expression, compared to effect observed by removal of H3K9me3 or over-expression of *LmPf2*, individually
- 20 out of 36 effector-genes associated with H3K9me3 are up-regulated in the
- Among the 256 effector-genes over-expressed naturally *in planta* in the WT, 45% were up-regulated in at least one of the transformants, 34% in the $\Delta KMT1$ _oPf2 transformants

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06/ Take-home message & future work

- KMT1 inhibits expression of genes naturally over-expressed *in planta*, *LmPf2* positively regulates these genes
- Regulation of the expression of effector-genes is a two layer-mechanisms
- What are the direct targets of *LmPf2*?