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Regulation of effector-gene expression as concerted waves in L. maculans: a two-player game

C. Clairet, E.J. Gay, A. Porquier, T. Rouxel, M-H. Balesdent, I. Fudal, J.L. Soyer INRAE, UR BIOGER, Université Paris-Saclay, Palaiseau, France



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



Successives 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.

• 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 plantpathogenic fungi

Our working hypothesis: a two-layer regulatory network

In vitro growth

In planta infection



Effector-genes are repressed due

to their localisation in H3K9me3-

or H3K27me3-domains

Effector-genes are expressed in finetuned successive waves due to removal of H3K9me3 and action of one / several TFs (transcription factors)

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

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

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

02/ Methodolody

- 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 Δ*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





- *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

genesª

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

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



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.

name	TEª	H3K4me2⁵	H3K9me3⁵	H3K27me3⁵	∆kmt1°	∆LmPf2 ^c	JN2_o <i>Pf2</i> °	⊿kmt1_o <i>Pf</i> 2⁵
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
AvrLmS-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

H3K4me2 H3K9me3 H3K27me3 H3K9me3/H3K27me3 Effector genes genes^a genes^a genesª

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

Up- <u>regulated</u>	∆kmt1	129	22*	22 [#]	3	64*	4*
	∆LmPf2	202	20	68 [#]	4	57*	5*
	WT_oPf2	247	46*	46 [#]	2	101*	6*
	∆kmt1_oPf2_B	931	154*	233 [#]	25*	329*	24*
	∆kmt1	143	27*	35 [#]	10*	42*	9*
Down-	∆LmPf2	225	40*	58 [#]	4	88*	10*
regulated	WT_oPf2	277	19	110 [#]	1	60*	3
	∆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 ChI², P value < 0.05. Carbohydrate uptake Oxydo-reduction processes

06/ Take-home message & future work

Total

genes

KMT1 inhibits expression of genes naturally over-expressed in planta, LmPf2 positively regulates these gene

- Regulation of the expression of effector-genes is a two layer-mechanisms
- What are the direct targets of LmPf2?

• 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 ΔKMT1_oPf2 transformants

	<u>Contact</u>	
	Jessica Soyer: jessica.	soyer@inrae.fr
	INRAE - UR BIOGER	
	Campus Agro Paris-Sa	clay
	22 Place de l'Agronon	nie
	91120 Palaiseau	References
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