

1 ***In planta* chromatin immunoprecipitation in *Zymoseptoria tritici* reveals chromatin-**
2 **based regulation of putative effector gene expression**

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21 Summary

22 During infection, pathogens secrete effectors, key elements of pathogenesis. In several
 23 phytopathogenic fungi, synchronous waves of effector genes are expressed during plant
 24 infection to manipulate and silence plant defenses. In *Zymoseptoria tritici*, causing
 25 septoria leaf blotch of wheat, at least two waves of effector genes are expressed, during
 26 the asymptomatic phase and at the switch to necrotrophy. The underlying factors
 27 responsible for the fine-tuned regulation of effector gene expression in this pathogen are
 28 unknown. Previously, a detailed map of the chromatin structure *in vitro* of *Z. tritici* was
 29 generated by chromatin immunoprecipitation followed by high-throughput sequencing
 30 (ChIP-seq) targeting histone modifications typical for euchromatin (di-methylation of the
 31 lysine 4 of the histone H3, H3K4me2) or heterochromatin (tri-methylation of the lysine 9
 32 and 27 of the histone H3, H3K9me3 and H3K27me3). Based on the hypothesis that
 33 changes in the histone modifications contribute to the transcriptional control of
 34 pathogenicity-related genes, we tested whether different sets of genes are associated
 35 with different histone modifications *in vitro*. We correlated the *in vitro* histone maps with
 36 *in planta* transcriptome data and show that genes located in heterochromatic domains *in*
 37 *vitro* are highly up-regulated at the switch toward necrotrophy. We combined our
 38 integrated analyses of genomic, transcriptomic and epigenomic data with ChIP-qPCR *in*
 39 *planta* and thereby provide further evidence for the involvement of histone modifications
 40 in the transcriptional dynamic of putative pathogenicity-related genes of *Z. tritici*.

41

42 Introduction

43 Fungi that colonize plant tissues, pathogens as well as mutualists, express effector genes
 44 required for their establishment within the plant tissue (Lo Presti *et al.*, 2015; Tyler and
 45 Rouxel, 2013). Transcriptomic analyzes have shown that effector genes are poorly
 46 expressed during axenic growth and strongly induced during host infection with several
 47 waves of concerted expression associated to infection stages (e.g. in *Melampsora larici-*
 48 *populina*, *Colletotrichum* species, *Leptosphaeria maculans*; Gervais *et al.*, 2017; Hacquard
 49 *et al.*, 2012; Lorrain *et al.*, 2018; O'Connell *et al.*, 2012). The fine-tuned regulation of
 50 effector genes is important for successful plant infection by fungi. However, very little is
 51 known about the underlying mechanisms that regulate expression of effector genes
 52 during different stages of host infection.

53 Transposable element (TE) rich regions of the genome are often enriched in genes
 54 encoding proteinaceous effectors or secondary metabolites (Soyer *et al.*, 2015a). In the
 55 oilseed rape pathogen *L. maculans*, effector genes expressed during infection of young
 56 leaves are enriched in TE-rich regions while effector genes expressed during stem
 57 infection are enriched in gene-rich regions of the genome (Gervais *et al.*, 2017).

58 In *L. maculans* and in the distantly related fungi *Epichloe festucae*, a symbiont of the grass
 59 *Lolium perenne*, it has been shown that histone modifications play a crucial role in the
 60 regulation of genes located in TE-rich regions involved in the establishment of fungus-
 61 plant interactions (Chujo and Scott 2014; Soyer *et al.*, 2014). These studies support the
 62 hypothesis that chromatin-mediated gene regulation represents an efficient strategy to

63 control genes located in the vicinity of TEs.

64 *Zymoseptoria tritici* causes the septoria tritici blotch disease on wheat (*Triticum aestivum*)
 65 (O'Driscoll *et al.*, 2014). Infection conferred by *Z. tritici* is initiated by a asymptomatic
 66 phase during which the pathogen grows intercellularly. Ten to 12 days post infection, the
 67 fungus switches to a necrotrophic stage inducing host cell death. The release of nutrients
 68 from dead plant cells allows the formation of pycnidia and the production of asexual
 69 spores responsible for new infections. Thus, successful infection of *Z. tritici* involves a
 70 lifestyle transition conferred by distinct transcriptional programs (Haueisen *et al.*, 2018;
 71 Rudd *et al.*, 2015; Palma-Guerrero *et al.*, 2016). So far, little is known about the regulation
 72 of the different infection programs set up by this fungus.

73

74 The genome of *Z. tritici* comprises 13 core chromosomes and a variable number of
 75 accessory chromosomes showing presence-absence polymorphisms in different isolates
 76 (Wittenberg *et al.*, 2009; Goodwin *et al.*, 2011; Möller *et al.*, 2018). TEs represent 18% of
 77 the genome with a considerably higher abundance on the accessory chromosomes than
 78 on core chromosomes (Dhillon *et al.*, 2014; Grandaubert *et al.*, 2015). Transcriptomic
 79 analyses revealed a dramatic difference in the expression of genes located on core and
 80 accessory chromosomes, with the latter showing no or very low expression of most genes
 81 *in vitro* as well as *in planta* (Kellner *et al.*, 2014; Haueisen *et al.*, 2018).

82 In a previous study, we performed chromatin immunoprecipitation followed by high-
 83 throughput sequencing (ChIP-seq) *in vitro* to determine the genome-wide location of
 84 histone modifications typical for euchromatin (di-methylation of the lysine 4 of the

histone H3, H3K4me2) or with heterochromatin (tri-methylation of the lysines 9 and 27 of H3, H3K9me3 and H3K27me3) (Schotanus *et al.*, 2015). In *Z. tritici*, TE-rich regions are enriched with both heterochromatic marks, H3K9me3 and H3K27me3. Furthermore, we confirmed that transcriptional silencing of genes on the accessory chromosomes is due to the strong enrichment with heterochromatic DNA as these chromosomes are almost entirely associated with H3K27me3 (Schotanus *et al.*, 2015).

Here, we investigated whether a chromatin-based control plays a role in the transcriptional regulation of pathogenicity-related genes in *Z. tritici*. We combined and integrated genomic, transcriptomic and epigenomic data and performed ChIP followed by quantitative PCR (ChIP-qPCR) analysis of targeted genes during plant infection. We provide evidence for an involvement of H3K9/K27me3 dynamics in the transcriptional regulation of putative pathogenicity genes of *Z. tritici*.

Results

Distribution of histone modifications in the coding fraction of the *Zymoseptoria tritici* genome

The ChIP-seq datasets generated from axenic cultures of the isolate Zt09 were analyzed to identify the genes associated with H3K4me2, H3K9me3 or H3K27me3. As previously described, H3K4me2 is mainly associated with gene-rich regions while the heterochromatin mark H3K9me3 is associated with the repetitive regions of the *Z. tritici* genome; H3K27me3 is likewise associated with repetitive sequences, telomeric regions,

accessory chromosomes, and with some gene coding sequences on core chromosomes.

We used the genome wide maps of H3K4me2, H3K9me3 and H3K27me3 from *in vitro* growth to distinguish genes either entirely or partially (> 1 bp) associated with each of the three histone modifications. Forty-two percent of the predicted genes in the genome are either associated with H3K4me2 (4,992 out of the 11,754 genes predicted in the genome), 0.75% (89 genes) with H3K9me3, 18.5% (2,179 genes) with H3K27me3 and 1.95% (230 genes) with both H3K9me3 and H3K27me3 (Figure 1A and 1B).

Genes located in H3K9me3 and H3K27me3 domains are less conserved and encode proteins putatively involved in virulence

We assessed the correlation of chromatin marks with the distribution of specific-specific genes previously identified in *Z. tritici* (Grandaubert *et al.*, 2015). Using comparative genomics of four *Zymoseptoria* species, i.e., *Z. tritici*, *Zymoseptoria pseudotritici*, *Zymoseptoria ardabiliae* and *Zymoseptoria brevis*, we previously identified 1,717 genes unique to *Z. tritici*. We found that these genes are significantly enriched in regions of the genome that are associated with H3K27me3 domains and overlapping H3K9me3/H3K27me3 domains: in total 951 of the *Z. tritici* species-specific genes locate in heterochromatic domains (X^2 test, $P < 0.01$; Table 1; Table S1). The *Z. tritici* genome encodes 874 putative effector genes and, interestingly, the species-specific genes are enriched in genes encoding putative effectors (177 representing 10.3% of the orphan genes; X^2 test; $P < 2.2 \cdot 10^{-6}$) of which 82 locate in H3K9me3 and / or H3K27me3 domains.

In the *Z. tritici* reference genome, 6,329 predicted genes could not be assigned to a protein function (i.e. no homology with proteins predicted in other organisms and/or no protein domain included in databases). Hereinafter, we refer to these as genes of "unknown function". We found a significant enrichment of genes of unknown function in the heterochromatic domains enriched with H3K27me3 or enriched with both H3K9me3 and H3K27me3 (χ^2 test, $P < 0.01$; Table 1; Table S2). Contrarily, genes associated with H3K4me2 were deprived of genes of unknown function compared to the rest of the genome (χ^2 test, $P < 0.01$; Table S2). Taken together, these data suggest that heterochromatic DNA associates with less conserved genes, including many predicted to be secreted and involved in host-pathogen interactions.

H3K9me3 and H3K27me3 are associated with genes encoding putative pathogenicity determinants

Specific types of proteins are often associated with pathogenicity in fungi (for example proteinaceous effectors and proteins involved in secondary metabolite syntheses; Tyler and Rouxel, 2013). In some species, such as *Aspergilli*, secondary metabolite-encoding genes were found to be enriched in subtelomeric regions of the genome or near TEs (Gacek and Strauss, 2012). Furthermore, a chromatin-based regulation of these genes by post-translational histone modifications was demonstrated for gene clusters encoding polyketide synthase-like (PKS) proteins and non-ribosomal peptide synthase (NRPS) gene clusters, e.g. in the endophyte *E. festucae* (Chujo and Scott, 2014).

We assessed whether PKS and NRPS encoding genes are associated with the two heterochromatin marks studied here. In total, 2,498 predicted genes (21% of all genes) locate in heterochromatic domains during *in vitro* growth of *Z. tritici*. Among these, we identified several genes involved in secondary metabolism and detoxification, i.e. with a predicted function such as cytochrome P450, polyketide synthase or Major Facilitator Superfamily (MFS) transporters. More precisely, 11 genes of the *Z. tritici* genome were annotated as PKS and nine as NRPS encoding genes (Ohm *et al.*, 2012; Grandaubert *et al.*, 2015). PKS/NRPS encoding genes are significantly enriched in H3K27me3 domains as identified during *in vitro* growth of the fungus (χ^2 test, $P < 0.01$, Table 1; Table S1).

We next used a PFAM enrichment analysis (Finn *et al.*, 2014) to assess if genes encoding proteins involved in biosynthetic activities were also enriched in heterochromatic domains of the *Z. tritici* genome. We found that H3K27me3 and H3K9me3 domains are significantly enriched in genes involved in biosynthetic activities including genes encoding Rad51 PFAM domains known to be involved in DNA repair processes and genes encoding reverse transcriptases and transposases. Furthermore, genes located in H3K27me3 domains are enriched in PFAM protein families involved in secondary metabolism (Tables S3-6; FDR < 0.01).

We also analyzed the location of 227 genes predicted to encode Carbohydrate-Active Enzymes (CAZymes) in the *Z. tritici* genome. Contrary to secondary metabolite genes, there is no significant enrichment of the CAZyme genes in heterochromatic domains (χ^2 test, $P > 0.01$; Table 1; Table S2).

In the pathogenic fungus *L. maculans*, effector genes showing stage-specific expression profiles during infection of oilseed rape (*Brassica napus*), are enriched in GC-poor or TE-rich genomic regions (Gervais *et al.*, 2017). Interestingly, the silencing of genes located in TE-rich regions, *in vitro*, involves a chromatin-based control via H3K9me3 (Soyer *et al.*, 2014). We addressed whether TE-rich genomic regions and heterochromatic domains of the *Z. tritici* genome likewise are enriched in putative effector genes. The 874 predicted effector genes represent eight percent of all predicted genes in *Z. tritici* and only nine on the accessory chromosomes (1.04% of all genes predicted on the accessory chromosomes). We here refined our analyses to address the association of the 865 putative effector genes of the core chromosomes and TEs. We considered a gene to be associated with TE regions if the gene locates within 2 kb distance of a TE. Assigning this criterion, we confirm that TE-rich regions of core chromosomes are enriched in putative effector genes (185 putative effector genes corresponding to 12.6% of the TE associated genes; χ^2 test, $P < 0.01$; Table 2; Table S7; Figure 2). We also found a significant enrichment of putative effector genes in heterochromatic domains associated with TEs or not (H3K9me3, H3K27me3 and H3K9me3/H3K27me3 overlapping domains; Table 2; Table S7) while euchromatic domains are deprived of putative effector genes (χ^2 test, $P < 0.01$; Table S7). Altogether, our analysis suggests that genes encoding putative pathogenicity-determinants are non-randomly distributed in the genome of *Z. tritici* and in particular enriched in H3K27me3 and H3K9me3 domains *in vitro*.

In vitro heterochromatic domains are enriched with genes up-regulated upon plant infection

As genes predicted as putative pathogenicity determinants (PKS, NRPS, effector genes) appear not to be randomly located in the genome of *Z. tritici*, we hypothesize that regulation of the expression of these genes involves a chromatin-based control as shown for effectors in *L. maculans* (Soyer *et al.*, 2014). We therefore assessed the correlation of the genome-wide expression patterns in the *Z. tritici* isolate Zt09 with the distribution of genome-wide map of H3K4me2, H3K9me3 and H3K27me3 histone modifications observed during *in vitro* growth (Schotanus *et al.*, 2015). To this end, we processed transcriptomic data generated from axenic cultures and infected plant material from previous studies (Kellner *et al.*, 2014, Haueisen *et al.*, 2018).

During axenic growth, 9,412 genes are expressed with RPKM ≥ 2 . Ninety-eight percent (3,237) of the genes for which sequence is entirely associated with H3K4me2, 58% and 39% of genes (i.e. 18 and 737 genes) associated with H3K9me3 or H3K27me3 respectively were expressed *in vitro* (RPKM ≥ 2). These patterns confirm that H3K4me2 is almost systematically associated with transcriptional activity while H3K9me3 and H3K27me3 represent repressive histone modifications (Wilcoxon-test, $P < 0.05$; Figure 3).

We then compared the transcriptomic profiles of Zt09 genes during host infection and assessed genome wide expression patterns at 4 and 13 days post inoculation (dpi), i.e. during early asymptomatic host infection and at the transition to necrotrophic growth, respectively. We analyzed 1) the number and distribution of genes silenced during *in vitro*

growth and induced during plant infection and 2) the number and distribution of genes significantly up- or down- regulated *in planta* compared to axenic culture (Figure 4).

We first analyzed genes specifically induced at 4 or 13 dpi compared to axenic culture (i.e. $RPKM \leq 2$ for the axenic culture and $RPKM \geq 2$ at 4 or 13 dpi). Using these criteria we found 559 genes induced at 4 dpi, including 92 genes that locate in an *in vitro* H3K4me2 domain and 149 in an *in vitro* H3K27me3 domain (the H3K4me2 domains comprise a total of 4,992 genes and H3K27me3 a total of 2,179 genes) (Table 1; Table S2). This pattern shows that H3K27me3 domains are enriched in genes specifically expressed at 4 dpi compared to axenic culture (X^2 test, $P < 0.01$; Table 1; Table S2). At 13 dpi, 886 genes, not expressed *in vitro*, are induced (i.e. $RPKM \leq 2$ for the axenic culture and $RPKM \geq 2$ at 13 dpi), including 98 genes located in an *in vitro* H3K4me2 domain and 355 genes located in an *in vitro* H3K27me3 domain. For this later stage of infection, as for 4 dpi, we also found that genes specifically expressed at 13 dpi compared to axenic culture are enriched in genes associated with *in vitro* H3K27me3 domains (X^2 test, $P < 0.01$; Table 1; Table S2).

We next analyzed genes up-regulated *in planta* compared to the axenic culture ($\log_2$ Fold Change ($RPKM$) ≥ 1 and $FDR \leq 0.001$). At 4 dpi, 784 genes were significantly up-regulated compared to the axenic growth. These up-regulated genes are significantly enriched in genes encoding effector candidates (114 putative effector genes of all up-regulated genes at 4 dpi; X^2 test, $P < 0.01$; Table 2; Table S7). However, during early infection, up-regulated genes are not significantly associated with any of the histone modifications investigated here. At 4 dpi, there is no effect of the *in vitro* location in H3K4me2, H3K9me3 or

H3K27me3 on the up- or down-regulation of genes (Table 1; Table S2; Figure 4A). At 13 dpi, 1,773 genes were specifically up-regulated compared to 4 dpi, including 388 genes and 507 genes located respectively in a H3K4me2 and in a H3K27me3 domain *in vitro*. As for 4 dpi, up-regulated genes were significantly enriched in effector genes (309 putative effector genes; χ^2 test, $P < 0.01$; Table 2, Table S7). At 13 dpi, transcription of the genes located in an *in vitro* heterochromatic domain is significantly different from genes located in an *in vitro* euchromatic domain (Wilcoxon test, $P < 2.2 \cdot 10^{-16}$; Figure 4B). We observe that genes showing low, or no, expression *in vitro* and associated with a histone modification typical for heterochromatin in this growing condition are significantly higher expressed at the switch to necrotrophy than genes associated *in vitro* with euchromatin (Table 1; Table S2). Up-regulated effector genes were likewise enriched in genes located in the vicinity of TEs (i.e. distance ≤ 2 kb) and H3K27me3 *in vitro* (Table 2; Table S7). Taken together, these data show that although sets of putative effector genes were highly expressed both 4 dpi and 13 dpi, the histone modifications associated with the genes possibly influences their expression only at the switch to necrotrophy.

KMT1, KMT6 or KMT1/KMT6 deletions deregulate expression of pathogenicity-related genes

In *Z. tritici*, *kmt1*, *kmt6* and *kmt1/kmt6* mutants were generated and gene expression was analyzed, *in vitro*, using RNA-seq (Moeller *et al.*, 2018). We processed these data to address whether KMT1 and / or KMT6 may influence pathogenesis-related genes.

253 Among the 100 most up-regulated genes in respectively the *kmt1*, *kmt6* and *kmt1/kmt6*
254 mutants, 59, 36 and 32% are over-expressed at 13 dpi compared to axenic culture in the
255 WT strain suggesting that both histone methyltransferases are involved in regulation of
256 genes highly expressed in the WT strain upon infection. We analyzed whether certain
257 categories of genes were significantly enriched among the deregulated genes in the
258 mutant background (Table 3). *KMT1* deletion induces the reorganization of H3K27me3
259 modification with a relocation of this modification to H3K9me3 regions (Moeller *et al.*,
260 2018). Hence, genes located in H3K9me3 are not significantly induced because of
261 H3K9me3 loss as they may still be repressed by the H3K27me3 modification (Table 3).
262 However, consistently with the relocation of H3K27me3, genes located in a H3K27me3
263 domain in the WT are significantly induced in the *kmt1* mutant (Table 3). We identified
264 that deregulated genes in the *kmt1*, *kmt6* or *kmt1/kmt6* mutants were significantly
265 enriched in SP-genes (i.e. 220 putative effectors within the 2,110 deregulated genes) and
266 genes associated with TE sequences while no significant association of the CAZymes
267 encoding genes was observed (Table 3; χ^2 test, $P < 0.01$). Particularly, the 972 genes up-
268 regulated in at least one of the mutants include 118 putative effector genes. Deletions of
269 *KMT1* and *KMT6* also significantly influenced expression of genes otherwise found to be
270 induced during infection in the WT strain (Table 3). Genes up-regulated at 4 dpi compared
271 to axenic culture, or 13 dpi compared to 4 dpi, in the WT strain are also significantly
272 enriched in the deregulated genes in the different mutants (231 out of the 784 genes up-
273 regulated at 4 dpi and 646 out of the 1,773 genes up-regulated at 13 dpi are deregulated
274 in the mutants).

We analyzed the expression of a few randomly selected genes based on their location in a heterochromatin domain *in vitro*, by RT-qPCR, in *kmt1*, *kmt6* and *kmt1/kmt6* deletion mutants. Some of these genes are putative effector genes or orphan genes and some are up-regulated in the WT at 13 dpi compared to early infection (4 dpi) in wheat (Table 4). One gene located in a H3K27me3 domain was not influenced by none of the deletion (gene ID Zt09_chr_3_00231) while all other genes had their expression up-regulated due to the deletion of at least *KMT1*, *KMT6* or both.

Up-regulation of effector candidate genes *in planta* is associated with changes in chromatin structure

The analysis of *in vitro* ChIP-seq data and *in planta* RNA-seq data indicates that chromatin modifications might contribute to the expression of pathogenicity related genes. *KMT1* and *KMT1/KMT6* deletions resulted in impaired *in vitro* growth and host colonization is consistently reduced compared to the WT while *KMT6* deletion only slightly reduced infection abilities. In all deletion backgrounds no large effect on gene expression was observed (Moeller *et al.*, 2018). Analyses of gene expression in *KMT1* or *KMT6* mutants appear not to be the most appropriate approach to disentangle influence of histone modifications on the regulation of pathogenicity related genes. Therefore, we further investigated histone modification dynamics, *in planta*, in the WT strain. We investigated whether the up-regulation of putative effector genes at 13 dpi is associated with a difference in the histone methylation patterns between *in vitro* and *in planta* growth. We compared the histone methylation patterns in the genomic sequence of different genes

296 that are lowly expressed *in vitro* and up-regulated during the lifestyle switch *in planta*,
 297 using ChIP-qPCR. The histone H4 encoding gene, constitutively expressed, and a
 298 transposable element, constitutively silenced (the DNA transposon, DTH_element
 299 5_ZTIPO323, located on chromosome 9;) were used as controls in the experiment.

300 The three selected genes (Zt09_chr_5_00271; Zt09_chr_6_00192; Zt09_chr_9_00038)
 301 encode putative effectors among the 10 most expressed genes in *Z. tritici* at 13 dpi and
 302 these are considerably less expressed *in vitro* compared to the histone H4 gene (Table
 303 S8). The putative effector gene Zt09_chr_9_00038 encodes a hydrophobin-like protein
 304 considered to be a “core” necrotrophic effector, i.e. conserved in different isolates of *Z.*
 305 *tritici* (Haueisen *et al.*, 2018). Using ChIP-qPCR, we confirm the *in vitro* association of the
 306 five genes with H3K4me2, H3K9me3 or H3K27me3 ("% of input" method). Consistently,
 307 the histone H4 encoding gene was associated with H3K4me2 while the TE sequence was
 308 associated with H3K9me3 and H3K27me3 and the three putative effector genes were
 309 located in heterochromatic domains (H3K9me3 or H3K27me3) *in vitro* (Figure 5; Table
 310 S8).

311 For the *in planta* material, we confirmed the chromatin immunoprecipitation by a qPCR
 312 targeting the wheat Actin gene (Genebank accession number KC775780.1, cultivar BR34)
 313 at 13 dpi. We validated that there was no product for the wheat actin primers in the ChIP
 314 samples generated from fungal axenic culture. The abundance of H3K4me2 for the wheat
 315 actin gene was considerably higher compared to levels of H3K9me3 and H3K27me3
 316 (Figure S1). In order to compare values obtained for the fungal genes *in vitro* and *in planta*,

we calculated enrichment values of the targeted genes relatively to the fungal histone H4 gene. We firstly confirmed that this method led to the same conclusion as the "% input" method (Figure 5 and Table S8). Furthermore, we checked the difference in terms of enrichment between the gene promoter and the coding sequence: we applied qPCR following ChIP targeting the upstream gene sequence and the coding region of the gene Zt09_chr_6_00192. The levels of H3K4me2, H3K9me3, H3K27me3 shows the same profile across the promoter and coding sequence, i.e. level of any histone modifications is similarly high or low for the upstream gene sequence or the coding region of the gene (Figure 6) between the promoter and the coding sequence of a gene.

At 13 dpi, the three targeted effector genes are highly expressed (Table S8) and the relative enrichment of H3K4me2 remained low compared to histone H4 gene (% of H3K4me2 enrichment for these genes compared to histone H4 < 0.2). On the contrary, the levels of H3K9me3 decreased (a relative 3-fold lower amount of H3K9me3) for the genes Zt09_chr_5_00271 and Zt09_chr_9_00038. For two genes located in H3K27me3 *in vitro* (Zt09_chr_9_00038 and Zt09_chr_6_00192), levels of H3K27me3 were 6-22 fold lower at 13 dpi compared to axenic culture. This suggests that the up-regulation of these genes at 13 dpi is associated with a remodeling of the underlying chromatin structure in their genomic sequence (Figure 6) and that the influence of the chromatin structure on effector gene expression is due to a decrease of the heterochromatin marks rather than an increase of the euchromatic mark investigated here.

We have assessed the levels of H3K4me2, H3K9me3 and H3K27me3 for the TE relatively

to the histone H4 gene: levels of H3K9me3 and H3K27me3 decreased compared to the levels *in vitro* but remained very high when compared to histone H4 and the three tested putative effector genes, which are up-regulated at this stage. Assessment of the chromatin state at the genomic loci of these genes confirms that constitutively expressed genes, such as the histone H4 gene, remain associated with the euchromatic modification H3K4me2 while the expression changes of the three effector genes tested are associated with changes of the associated histone modifications during wheat infection. This data provide evidence of an involvement of chromatin dynamics for the *in planta* regulation of secreted protein encoding genes putatively involved in pathogenicity.

Discussion

In some plant pathogenic fungi, including *Z. tritici*, the expression profiles of effector genes was shown to be highly dynamic during host infection. By correlating genomic coordinates of predicted effector genes to transcriptome and epigenome data, we here provide evidence for a role of a chromatin-based gene regulation during plant infection of *Z. tritici*. In particular, candidate effector genes up-regulated during the transition from asymptomatic to necrotrophic growth are significantly enriched in regions of the genome that are transcriptionally silenced by a heterochromatin structure of the DNA during axenic growth. In this study, we analyzed levels of different histone modifications typical for heterochromatin or euchromatin *in planta* by applying ChIP-qPCR targeting the genomic loci of three effector genes that are among the most up-regulated genes at 13

dpi. Our experimental work provides evidence that histone modifications H3K9me3 and H3K27me3 play a role for the transcriptional regulation of putative effector genes in *Z. tritici* during wheat infection. Our targeted analysis *in planta* thereby suggests that the dynamic expression of effector genes can be associated with a dynamic of the heterochromatin marks H3K9me3 and H3K27me3.

Genes located in a heterochromatin domain in vitro are enriched in putative virulence related genes

In other species, such as *L. maculans*, *Fusarium oxysporum*, Aspergilli species, genes located in regions that are TE-rich, subtelomeric, lineage-specific, are often enriched in genes encoding putative effectors or genes involved in secondary metabolite production (Rouxel *et al.*, 2011; Grandaubert *et al.*, 2014; Ma *et al.*, 2010; Faino *et al.*, 2016; Vlaardingerbroek *et al.*, 2016). We find here that TE-rich genomic compartments of *Z. tritici* encode species-specific genes and genes encoding putative effector proteins. The fact that TE-rich regions are enriched in species-specific genes was also shown at the within species level (Plissonneau *et al.*, 2016). Interestingly, while the *Z. tritici* reference strains presents eight accessory chromosomes which contain twice as much TEs as the core chromosomes, they are not enriched in putative secreted protein encoding genes as only nine are located on the accessory chromosomes. Genes encoding other types of pathogenicity related genes such as PKS and NRPS were also enriched in H3K27me3 domains of core chromosomes. Together, these data show that in *Z. tritici*, pathogenicity-

related genes and genes involved in host specificity are enriched in repeat-rich, heterochromatic regions on the core chromosomes.

Genes highly expressed at the lifestyle switch towards necrotrophy are enriched in genes located in heterochromatin in vitro and in effector candidates

Using our previous RNA-seq data generated *in vitro* and at 4 and 13 dpi (Kellner *et al.*, 2014; Haueisen *et al.*, 2018), we investigated patterns of gene expression as a function of their location in euchromatin or a heterochromatin domain *in vitro*. Genes over-expressed at 4 dpi compared to axenic culture and at 13 dpi compared to 4 dpi are enriched in putative pathogenicity-related genes. Interestingly, we found that genes highly expressed at the onset of the infection are not enriched in *in vitro* H3K4me2, H3K9me3 or H3K27me3 domains. However, genes highly expressed at the switch to necrotrophic growth are, *in vitro*, associated with TE sequences and H3K9me3 and H3K27me3. The different relevance of H3K9me3 and H3K27me3 at the two studied infection stages suggests that the pattern of histone modifications is dynamic during infection, and possibly influenced by signals from the host tissue and / or by fungal development *in planta*. It is possible that transcription of the genes highly expressed during the second wave of effector gene expression remains suppressed during early host infection to avoid induction of host immune responses. During early host infection, specific effectors are likely relevant to suppress the plant immune system upon host penetration and biotrophic fungal establishment.

Recently, functional analyses in *Z. tritici* have identified two genes encoding avirulence proteins (i.e., effector that can be recognized by the immune system of the plant activating defense reactions): *Avr3D1* and *AvrStb6*, over-expressed during wheat infection just before the switch to necrotrophic growth (Zhong *et al.*, 2017; Meile *et al.*, 2018). In our ChIP-seq data, *AvrStb6*, located in a subtelomeric region, is associated with H3K9me3 and H3K27me3 while *Avr3D1*, located close to repetitive sequences, is associated with H3K27me3. Some of the genes encoding candidate necrosis inducing proteins, highly expressed at the switch to necrotrophy, identified in a previous study (Ben M'Barek *et al.*, 2015) are also associated with heterochromatin domains *in vitro*, for instance genes Zt09_chr_5_00190 or Zt09_chr_2_01243. The association of these genes with H3K27me3 or TEs and their expression patterns support our findings drawn at the genome wide scale.

The histone modification patterns is dynamic between in vitro and in planta stages for a few effector genes, influencing their expression

The role of the chromatin structure for the regulation of effector genes in TE-rich regions has been investigated in *L. maculans*, whereby RNAi-silenced transformants were generated for genes encoding HP1 and KMT1, two players involved in heterochromatin assembly and maintenance (Soyer *et al.*, 2014). In this fungus, silencing of *HP1* and *KMT1* led to an up-regulation of genes located in TE-rich regions, notably effector genes, correlated with a decrease in H3K9me3 (Soyer *et al.*, 2014). The same experimental

strategy was applied to investigate the role of histone modifications for the regulation of secondary metabolite-encoding genes in other fungal species including *F. graminearum*, *E. festucae* and Aspergilli species (Reyes-Dominguez *et al.*, 2012; Chujo and Scott, 2014; Gacek-Matthews *et al.*, 2015; Gacek-Matthews *et al.*, 2016). In *E. festucae*, ChIP-qPCR was also applied *in planta* to analyze histone modification patterns at the genomic loci of secondary metabolite gene clusters located in subtelomeric areas. In *Fusarium fujikuroi*, deletion of the H3K36 methyltransferase or *KMT6* resulted in deregulation (either up- or down-regulation) of some secondary metabolite gene clusters (Janevska *et al.*, 2018; Studt *et al.*, 2016). These studies, together with other analyzing gene expression at the genome-wide scale in plant pathogenic fungi (Connolly *et al.*, 2013; Soyer *et al.*, 2014; Moeller *et al.*, 2018) show that loss of histone methyltransferases result in derepression of some genes associated with the histone modifications while a fraction remains silenced suggesting that a very complex regulatory network is involved in their transcriptional control. As in Soyer *et al.* (2014), our analysis of gene expression in *KMT1*, *KMT6* mutants of *Z. tritici* (Moeller *et al.*, 2018; our study) has shown that pathogenicity-related encoding genes and genes expressed upon infection, are enriched within the deregulated genes due to at least one of the deletion. In order to analyze influence of histone modifications in the WT context and during plant infection, we applied for the first time ChIP-qPCR *in planta* to analyze levels of H3K4me2, H3K9me3 and H3K27me3 of three genes encoding effector candidates which are highly expressed at 13 dpi, as well as the constitutively expressed histone H4 gene and a transposable element sequence. We could demonstrate that levels of H3K4me2, H3K9me3 and H3K27me3 for each targeted loci correlated with

their expression *in vitro* and at 13 dpi. For the repetitive element, levels of H3K9me3 and H3K27me3 were high *in vitro* as well as *in planta* likely reflecting the efficient silencing of TEs in some regions of the genome. However, effector genes associated with a high level of H3K9me3 and H3K27me3 *in vitro* showed significantly reduced levels of these marks *in planta* consistent with their strong up-regulation. This indicates that the chromatin structure is dynamic between axenic culture and different infectious stages and instrumental for regulation of gene expression, notably of at least some effector genes in *Z. tritici*.

In conclusion, our study indicates a prominent role of chromatin-based gene regulation during wheat infection of *Z. tritici*. The close association of putative effector genes with repeat-rich DNA may facilitate rapid evolution of these genes. Furthermore, the association with heterochromatin in these genomic compartments provides variability at the transcriptional level possibly further facilitating the defeat of host defenses by this important wheat pathogen.

Experimental Procedures

Fungal strain and plant cultivar

The *Z. tritici* isolate Zt09 was used throughout the study. Zt09 is derived from the reference isolate IPO323, and differs by the absence of chromosome 18, lost during *in vitro* culture (Kellner *et al.*, 2014). Cultures were grown on solid yeast malt sucrose (YMS)

agar (4 g yeast extract, 4 g malt extract, 4 g sucrose, 20 g bacto agar, 1 liter H₂O) at 18°C in the dark. For the *in planta* experiments conducted in this study, *Triticum aestivum* cultivar Obelisk (Wiersum Plantbreeding, Winschoten, the Netherlands) was used. The *kmt1*, *kmt6* and *kmt1/kmt6* mutants were generated by Moeller *et al.* (2018).

Identification of genes associated with specific histone modifications

The annotation of the *Z. tritici* isolate presented in Grandaubert *et al.* (2015) was used in this study including genes predicted to encode putative effectors and Polyketide Synthases/NonRibosomal Peptide Synthetases. To distinguish genes associated with the different histone marks H3K4me₂, H3K9me₃ and H3K27me₃, we used previously generated *in vitro* ChIP-seq datasets (Schotanus *et al.*, 2015) available under the SRA accession number SRP059394. The Integrative Genome Viewer (IGV; <http://www.broadinstitute.org/software/igv>; Thorvaldsdóttir *et al.*, 2013) was used to visualize location of each domains along the genome. Statistically enriched regions were identified with RSEG (Song and Smith, 2011). We determined the *in vitro* chromatin state of each annotated gene using the previously published chromatin maps (Schotanus *et al.*, 2015). Genes were considered to be associated with a given post-translational histone modification when partially (at least one bp) or completely located in a H3K4me₂, H3K9me₃ or H3K27me₃ domain. A Chi² test was applied to identify statistical enrichment of euchromatic or heterochromatic domains for certain categories of genes: the expected proportion of a given category of genes across the entire genome was compared to the

observed distribution of the gene category in the H3K4me2, H3K9me3 or H3K27me3 domains. Enrichment was considered significant with a P value < 0.01 . All analyses were done in R (www.r-project.org).

RNA-sequencing datasets

To correlate gene expression with location in a given chromatin domain during *Z. tritici* infection of wheat, we used previously generated *in vitro* and *in planta* (four and 13 dpi) RNA-seq datasets of Zt09 (Kellner *et al.*, 2014; Haueisen *et al.*, 2018). RNA-seq data from the *in vitro* grown cultures and the *in planta* stages are accessible at the NCBI Gene Expression Omnibus respectively through accession number GSE54874 and GSE106136 (Kellner *et al.*, 2014; Haueisen *et al.*, 2018). RNA-seq data from the *kmt1*, *kmt6* and *kmt1/kmt6* mutants during *in vitro* growth were generated by Moeller *et al.* (2018) and at Sequence Read Archive under BioProject ID PRJNA494102.

RPKM values for each gene in a given condition were estimated using Cufflinks (Trapnell *et al.*, 2010) as already described in Kellner *et al.* (2014). The total read counts per transcript were estimated in HTSeq using the intersection-strict mode (Anders *et al.*, 2015) and genes differentially expressed were identified with the EdgeR package (Robinson *et al.*, 2010) with a log2 Fold Change ≤ -1 or ≥ 1 and an associated false discovery rate ≤ 0.001 (McCarthy *et al.*, 2012). As previously conducted in a study of gene expression in *Z. tritici* (Kellner *et al.*, 2014), we only included genes with an RPKM value ≥ 2 in the condition in which the gene is up-regulated.

506

507 Plant experiments for chromatin immunoprecipitation quantitative PCR analyses

508 Plant infections were done as previously described (Habig *et al.*, 2017). In brief, the
509 second leaves of wheat seedlings were inoculated with a solution containing 1.10^7
510 cells/ml supplemented with 0.1% Tween by brushing an area of seven cm after 11 days of
511 seedling growth. Plant material was harvested four and 13 dpi for ChIP-qPCR. As a control,
512 non-inoculated leaves were also harvested four and 13 dpi.

513

514 Chromatin immunoprecipitation (ChIP) and quantitative PCR (qPCR)

515 ChIP with antibodies against H3K4me2, H3K9me3 and H3K27me3 was performed on cells
516 from axenic cultures as previously described (Soyer *et al.*, 2015). For *in planta* ChIP, 12
517 infected leaves were harvested (i.e. three leaves and four technical replicates) at 13 dpi
518 and the same protocol was used except that “native ChIP” (without formaldehyde
519 crosslinking) was applied from 100 mg of infected plant material. All DNA extractions were
520 done with the Wizard® SV Gel and PCR Clean Up system kit (Promega, Madison, USA).

521 Based on our analyses of transcriptome, genome and ChIP-seq data, we selected three
522 candidate genes for ChIP-qPCR analyses: Zt09_chr_5_00271, Zt09_chr_6_00192,
523 Zt09_chr_9_00038. These genes are lowly expressed *in vitro* and among the 10 most
524 expressed genes in *Z. tritici* 13 dpi of wheat. Moreover, they are associated with
525 heterochromatin during *in vitro* growth (Table S8; Schotanus *et al.*, 2015). Furthermore,

we included the sequence of a transposable element (DTH_element5_ZTIPO323, located on chromosome 9, position 29,065-30,735) as well as the gene encoding histone H4 (ID Zt09_chr_6_00256) as controls for precipitation with anti-H3K9me3, anti-H3K27me3 antibodies and anti-H3K4me2 antibody, respectively.

Quantitative PCR (qPCR) was performed with SYBR Green PCR Master Mix (Applied Biosystem, Foster City, USA) on a 7900 Real Time PCR System (Applied Biosystems). Two biological replicates, and two technical replicates were processed. Primers were designed with the OligoPerfect Designer (ThermoFisher Scientific) to target amplification of products between 80 and 150 bp (Table S9). The efficiency of PCR primers was verified on genomic DNA as described previously (Taylor *et al.*, 2010). For RT-qPCR, Ct values were analyzed as described in Muller *et al.* (2002) for analysis of expression profiles and the Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)-encoding gene (ID Zt09_chr_2_00354) was used as a constitutively expressed reference gene, as in Poppe *et al.* (2015). A positive control for the *in planta* ChIP experiment was obtained by qPCR amplification of the wheat actin gene (Table S9). For qPCR after *in vitro* ChIP, the immunoprecipitated fraction of each gene was calculated by the “% of input” method (Lin *et al.*, 2012). Following the qPCR experiment, the Ct values (number of cycles required for the fluorescent signal to cross the threshold) were retrieved from the 7900 SDS software. To compare enrichment of the histone modifications H3K4me2, H3K9me3, and H3K27me3 for each target gene *in vitro* and *in planta*, we compared Ct values of the target genes to Ct values of a reference gene, histone H4 (i.e., for example, $\Delta Ct_{H3K4me2} = Ct_{\text{gene H3K4me2}} - Ct_{\text{histone H4 H3K4me2}}$). The same calculation was applied for the

histone H4 gene, therefore $\Delta Ct = 1$ for each modification for this gene. Finally, the enrichment of each target gene with the three histone modifications was calculated as described by Lin *et al.* (2012) using $\%H3K4me2 = 100/2^{\Delta Ct}$. This enrichment was compared for each gene *in vitro* and *in planta* (at 13 dpi).

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Author contributions

Conceived and designed the experiments: JLS, JG, EHS. Acquisition, analysis or interpretation of the data: JLS, JG, JH, KS, EHS. Writing of the manuscript: JLS, EHS.

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Figure legends

Figure 1. Euchromatic domains encompass more genes than heterochromatin domains *in vitro*. Venn diagrams showing genes associated with the different post-translational histone modifications assessed using ChIP-sequencing in axenic culture. A) Genes at least partially associated with any of the histone modification. B) Genes completely associated with any of the histone modifications.

Figure 2. Heterochromatic domains are enriched with putative effector genes that are up-regulated during the switch towards necrotrophic growth on wheat. Example from a section of chromosome 5, harboring the putative effector gene ID Zt09_chr_5_00271. Regions enriched in different histone modifications were identified using ChIP-seq with antibodies against H3K4me2 (light blue), H3K9me3 (purple), H3K27me3 (orange) and compared to the location of genomic features (coding sequences, dark blue; transposable elements, red) and to reads obtained by RNA-seq in axenic culture, 4 days post-infection (dpi) and 13 dpi.

Figure 3. Genes associated with euchromatin are expressed while genes associated with

heterochromatin are repressed. Expression assessed during axenic culture using RNA-sequencing. Boxplot of the $\log_{10}$ (RPKM) of the genes totally associated with any of the histone modification.

Figure 4. Genes associated with euchromatin and heterochromatin *in vitro* exhibit different expression profiles *in planta*. Boxplot of the $\log_{10}$ Fold Change (RPKM) of the genes located in a euchromatic domain *in vitro* (i.e. H3K4me2) or a heterochromatic domain *in vitro* (i.e. H3K9me3, H3K27me3 or H3K9me3/H3K27me3). Expression assessed during axenic culture and at 4 and 13 days post infection (dpi) using RNA-sequencing; genes differentially expressed at 4 dpi vs. axenic culture (A) or 13 dpi vs. 4 dpi (B). *: Wilcoxon-test, $P < 2.2 \cdot 10^{-16}$: comparison of heterochromatic-associated genes between 4 and 13 dpi.

Figure 5. Putative effector genes are enriched in H3K9me3 and/or H3K27me3 *in vitro* while histone H4 is enriched in H3K4me2. Chromatin immunoprecipitation was performed during axenic culture to assess the levels of H3K4me2, H3K9me3 and H3K27me3 in the genomic sequence of four genes (H4, Histone H4: Zt09_chr_6_00256; 5_00271: Zt09_chr_5_00271; 6_00192: Zt09_chr_6_00192; 9_00038: Zt09_chr_9_00038) and a transposable element (DTH_element5_ZTIPO323).

Figure 6. The up-regulation of three effector genes *in planta* is associated with a change of their histone modifications patterns. ChIP was performed at 13 days post infection of wheat leaves by *Zymoseptoria tritici* and relative enrichment of (A) H3K4me2, (B) H3K9me3 and (C) H3K27me3 was assessed using qPCR and compared during the axenic

813 culture and at 13 dpi. 5_00271: Zt09_chr_5_00271; 6_00192: Zt09_chr_6_00192;
814 6_00192 pro: Zt09_chr_6_00192, promoter; 9_00038: Zt09_chr_9_00038; K4, K9 and
815 K27: H3K4me2, H3K9me3 and H3K27me3 respectively. Blue: axenic culture; red: 13 dpi.

816

817 Table legends

818 **Table 1. Number of genes in a given category according to their location in a H3K4me2,**
819 **H3K9me3, H3K27me3 or H3K9me3/H3K27me3 domain *in vitro*.**

820 ^a“unknown” genes are genes encoding predicted or hypothetical proteins;

821 ^bGenes located up to 2 kb upstream or downstream to a transposable element sequence;

822 ^cGenes specifically expressed at 4 or 13 days post infection (dpi) compared to axenic
823 culture or 4 dpi when RPKM ≥ 2 at the time point and RPKM ≤ 2 during the axenic culture
824 or at 4 dpi;

825 ^dUp-regulated genes are genes with Log2 Fold Change (RPKM) ≥ 1 and FDR ≤ 0.001 in a
826 given condition compared to the other;

827 ^eDown-regulated genes are genes with Log2 Fold Change (RPKM) ≤ -1 and FDR ≤ 0.001 in
828 a given condition compared to the other;

829 ^fGenes located in a H3K4me2, H3K9me3, H3K27me3 or H3K9me3/H3K27me3 domain *in*
830 *vitro*.

831

832 **Table 2. Number of putative effector genes located in vicinity to a transposable element**
833 **or in a H3K4me2, H3K9me3, H3K27me3 and H3K9me3/H3K27me3 domain *in vitro*.**

Only putative effector genes located in the core genome of *Z. tritici* are shown here.

^aUp-regulated genes are genes with Log2 Fold Change (RPKM) ≥ 1 and FDR ≤ 0.001 in a given condition compared to the other;

^bDown-regulated genes are genes with Log2 Fold Change (RPKM) ≤ -1 and FDR ≤ 0.001 in a given condition compared to the other;

^cGenes located up to 2 kb upstream or downstream to a transposable element sequence;

^dGenes located in a H3K4me2, H3K9me3, H3K27me3 or H3K9me3/H3K27me3 *in vitro*.

Table 3. Influence of *kmt1*, *kmt6* or *kmt1/kmt6* deletions on gene expression.

^aGenes located in a H3K4me2, H3K9me3, H3K27me3 or H3K9me3, H3K27me3 *in vitro*;

^b“unknown” genes are genes encoding predicted or hypothetical proteins;

^cGenes located up to 2 kb upstream or downstream to a transposable element sequence;

^dGenes specifically expressed at 4 or 13 days post infection (dpi) compared to axenic culture or 4 dpi when RPKM ≥ 2 at the time point and RPKM ≤ 2 during the axenic culture or at 4 dpi;

^eUp-regulated genes are genes with Log2 Fold Change (RPKM) ≥ 1 and FDR ≤ 0.001 in a given condition compared to the other;

^fDown-regulated genes are genes with Log2 Fold Change (RPKM) ≤ -1 and FDR ≤ 0.001 in a given condition compared to the other;

^hGenes deregulated in *KMT1*, *KMT6* or *KMT1/KMT6* deletions background, Log2 Fold Change (RPKM) ≤ -1 or ≥ 1 and FDR ≤ 0.001 .

Bold: the given domain contains significantly less genes of the given category compared

to the rest of the genome; grey: the given domain is enriched for the category of genes compared to the rest of the genome.

Table 4. Expression of selected genes associated with heterochromatin *in vitro*, in the *KMT1*, *KMT6* and *KMT1/KMT6* deletion mutants.

^aGenes located up to 2 kb upstream or downstream to a transposable element sequence;

^bGenes located in a H3K4me₂, H3K9me₃, H3K27me₃ or H3K9me₃, H3K27me₃ *in vitro*;

^cGenes significantly up-regulated 13 days post-infection (dpi) compared to 4 dpi;

^dGene expression relative to the WT strain and to the GAPDH encoding gene.

Supplementary data legends

Figure S1. Wheat actin was enriched in histone H3K4me₂ and was used as a control for chromatin immunoprecipitation *in planta*. ChIP was performed at 13 days post infection of wheat leaves by *Zymoseptoria tritici*. Enrichment of the wheat actin gene in H3K4me₂, H3K9me₃ and H3K27me₃ was assessed using qPCR.

Table S1. Enrichment analysis of PKS/NRPS- and orphan genes in a H3K4me₂, H3K9me₃, H3K27me₃ or H3K9me₃/H3K27me₃ domain *in vitro*.

^aGenes encoding PKS, NRPS, and orphan genes as predicted by Grandaubert *et al.* (2015);

^bGenes with a RPKM ≥ 2 ;

^cGenes with Log₂ Fold Change (RPKM) ≥ 1 and FDR ≤ 0.001 at 4 days post infection (dpi)

compared to axenic culture or 13 dpi compared to 4 dpi were considered as up-regulated

at a given time point compared to the other. Only the genes with a RPKM ≥ 2 at least at

the time point during which it is up-regulated were kept for the analysis;

^dGenes with Log2 Fold Change (RPKM) ≤ -1 and FDR ≤ 0.001 at 4 days post infection (dpi)

compared to axenic culture or 13 dpi compared to 4 dpi were considered as down-

regulated at a given time point compared to the other;

^eGenes located in a H3K4me2, H3K9me3, H3K27me3 or H3K9me3/H3K27me3 *in vitro* as

identified with RSEG (see Experimental Procedures; Schotanus *et al.*, 2015).

Blue: the given domain contains significantly less genes of the given category compared

to the rest of the genome; orange: the given domain is enriched for the category of genes

compared to the rest of the genome.

Table S2. Enrichment analysis of genes of a given category in a H3K4me2, H3K9me3, H3K27me3 or H3K9me3/H3K27me3 domain *in vitro*.

^aGenes of “unknown function” are genes encoding predicted or hypothetical proteins;

genes encoding CAZymes as predicted by Grandaubert *et al.* (2015);

^bGenes located up to 2 kb upstream or downstream to a transposable element sequence

as predicted by Grandaubert *et al.* (2015);

^cGenes with a RPKM ≥ 2 ;

^dGenes with Log2 Fold Change (RPKM) ≥ 1 and FDR ≤ 0.001 at 4 days post infection (dpi)

compared to axenic culture or 13 dpi compared to 4 dpi were considered as up-regulated

at a given time point compared to the other. Only the genes with a RPKM ≥ 2 at least at

the time point during which it is up-regulated were kept for the analysis;

^eGenes with Log2 Fold Change (RPKM) ≤ -1 and FDR ≤ 0.001 at 4 dpi compared to axenic culture or 13 dpi compared to 4 dpi were considered as down-regulated at a given time point compared to the other;

^fGenes located in a H3K4me2, H3K9me3, H3K27me3 or H3K9me3/H3K27me3 *in vitro* as identified with RSEG (see Experimental Procedures; Schotanus *et al.*, 2015).

Blue: the given domain contains significantly less genes of the given category compared to the rest of the genome; orange: the given domain is enriched for the category of genes compared to the rest of the genome.

Table S3. PFAM analysis of the genes located in H3K4me2 domains *in vitro*.

Table S4. PFAM analysis of the genes located in H3K9me3/H3K27me3 domains *in vitro*.

Table S5. PFAM analysis of the genes located in H3K9me3 domains *in vitro*.

Table S6. PFAM analysis of the genes located in H3K27me3 domains *in vitro*.

Table S7. Enrichment analysis of putative effector genes in the vicinity of a transposable element or in a H3K4me2, H3K9me3, H3K27me3 and H3K9me3/H3K27me3 domain *in vitro*, and according to their expression.

^aGenes encoding predicted secreted proteins (SP) were considered as genes encoding

putative effector genes for this study, as predicted by Grandaubert *et al.* (2015);

^bGenes with Log2 Fold Change (RPKM) ≥ 1 and FDR ≤ 0.001 at 4 days post infection (dpi) compared to axenic culture or 13 dpi compared to 4 dpi were considered as up-regulated at a given time point compared to the other. Only the genes with a RPKM ≥ 2 at least at the time point during which it is up-regulated were kept for the analysis;

^cGenes with Log2 Fold Change (RPKM) ≤ -1 and FDR ≤ 0.001 at 4 dpi compared to axenic culture or 13 dpi compared to 4 dpi were considered as down-regulated at a given time point compared to the other;

^dGenes located up to 2 kb upstream or downstream to a transposable element sequence as predicted by Grandaubert *et al.* (2015);

^eGenes located in a H3K4me2, H3K9me3, H3K27me3 or H3K9me3/H3K27me3 *in vitro* as identified with RSEG (see Experimental Procedures; Schotanus *et al.*, 2015).

Blue: the given domain contains significantly less genes of the given category compared to the rest of the genome; orange: the given domain is enriched for the category of genes compared to the rest of the genome.

Table S8. Trimethylation of lysine 9 and / or 27 of histone H3 is reduced for three candidate effector genes up-regulated 13 days post infection of wheat leaves compared to axenic culture.

^aOrphan genes as described by Grandaubert *et al.* (2015);

^bGenes located at a distance below 2 Kb of a transposable element (Grandaubert *et al.*, 2015);

944 ^cRelative enrichment, *in vitro* or 13 dpi, of H3K4me2, H3K9me3 or H3K27me3 for the given

945 locus compared to histone H4 encoding gene (Zt09_chr_6_00256);

946 ^dGene expression *in vitro* (Kellner *et al.*, 2014);

947 ^eGene expression from Haueisen *et al.* (2018);

948 ^fExpression rank of the candidate genes at 13 days post infection (dpi).

949

950 **Table S9. List of primers used in this study.**

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952

953

Table 1. Number of genes in a given category according to their location in a H3K4me2, H3K9me3, H3K27me3 or H3K9me3/H3K27me3 domain *in vitro*.

	genome		H3K4me2-domains ^f		H3K9me3-domains ^f		H3K27me3-domains ^f		H3K9me3+H3K27me3-domains ^f	
	nb. in the entire genome	nb. in the core genome	nb. in the entire genome	nb. in the core genome	nb. in the entire genome	nb. in the core genome	nb. in the entire genome	nb. in the core genome	nb. in the entire genome	nb. in the core genome
total number of genes	11754	11111	4992	4991	89	89	2179	1661	230	171
unknown ^a	6329	5699	1944	1943	50	50	1734	1223	193	152
PKS/NRPS	20	20	1	1	0	0	10	10	0	0
CAZymes	227	227	58	58	4	4	32	32	3	3
genes < 2 Kb Tes ^b	1723	1464	369	368	83	83	545	358	202	154
orphan genes	1717	1221	177	177	21	21	794	392	136	91
genes expressed at 4 dpi vs axenic ^c	559	546	92	92	4	4	149	138	9	9
genes expressed at 13 dpi vs axenic ^c	886	778	98	98	10	10	355	253	20	17
genes expressed at 13 dpi vs 4 dpi ^c	729	577	53	53	10	10	368	224	27	20
genes up-regulated at 4 dpi vs. <i>in vitro</i> ^d	784	775	290	290	6	6	118	112	10	10
genes down-regulated at 4 dpi vs. <i>in vitro</i> ^e	1027	1001	379	379	3	3	179	156	23	21
genes up-regulated at 13 dpi vs. 4 dpi ^d	1773	1630	388	388	12	12	507	390	38	32
genes down-regulated at 13 dpi vs. 4 dpi ^e	798	796	385	385	5	5	81	80	12	11

^a“unknown” genes are genes encoding predicted or hypothetical proteins;

^bGenes located up to 2 kb upstream or downstream to a transposable element sequence;

^cGenes specifically expressed at 4 or 13 days post infection (dpi) compared to axenic culture or 4 dpi when RPKM ≥ 2 at the time point and RPKM ≤ 2 during the axenic culture or at 4 dpi;

^dUp-regulated genes are genes with Log2 Fold Change (RPKM) ≥ 1 and FDR ≤ 0.001 in a given condition compared to the other;

^eDown-regulated genes are genes with Log2 Fold Change (RPKM) ≤ -1 and FDR ≤ 0.001 in a given condition compared to the other;

^fGenes located in a H3K4me2, H3K9me3, H3K27me3 or H3K9me3/H3K27me3 domain *in vitro*.

Table 2. Number of putative effector genes located in vicinity to a transposable element or in a H3K4me2, H3K9me3, H3K27me3 and H3K9me3/H3K27me3 domain *in vitro*.

	core genome	TE-associated genes ^c	H3K4me2-domains ^d	H3K9me3-domains ^d	H3K27me3-domains ^d	H3K9+H3K27me3-domains ^d
total number of genes	11111	1464	4991	89	1661	171
putative effector genes	865	185	168	15	205	26
putative effector genes up-regulated at 4 dpi vs axenic ^a	114	14	24	3	22	1
putative effector genes down-regulated at 4 dpi vs axenic ^b	107	32	23	0	29	6
putative effector genes up-regulated at 13 dpi vs 4 dpi ^a	309	84	42	4	87	12
putative effector genes down-regulated at 13 dpi vs 4 dpi ^b	74	10	22	1	10	1

Only putative effector genes located in the core genome of *Z. tritici* are shown here.

^aUp-regulated genes are genes with Log2 Fold Change (RPKM) ≥ 1 and FDR ≤ 0.001 in a given condition compared to the other;

^bDown-regulated genes are genes with Log2 Fold Change (RPKM) ≤ -1 and FDR ≤ 0.001 in a given condition compared to the other;

^cGenes located up to 2 kb upstream or downstream to a transposable element sequence;

^dGenes located in a H3K4me2, H3K9me3, H3K27me3 or H3K9me3/H3K27me3 *in vitro*.

Table 3. Number of genes deregulated due to *KMT1*, *KMT6* or *KMT1/KMT6* deletions, *in vitro*.

	genome		<i>Δkmt1</i> ^h				<i>Δkmt6</i> ^h			
	nb. in the genome	proportion	up-regulated		down-regulated		up-regulated		down-regulated	
			nb. in the genome	proportion	nb. in the genome	proportion	nb. in the genome	proportion	nb. in the genome	proportion
total number of genes	11754		579		284		274		481	
H3K4me2 ^a	4992	0.425	116	0.200	71	0.250	92	0.336	194	0.403
H3K9me3 ^a	89	0.008	4	0.007	8	0.028	0		2	0.004
H3K27me3 ^a	2179	0.185	168	0.290	57	0.201	116	0.423	63	0.131
H3K9/K27me3 ^a	230	0.020	20	0.035	16	0.056	23	0.084	5	0.010
unknown ^b	6329	0.538	399	0.689	117	0.412	175	0.639	175	0.364
CAZymes	227	0.019	10	0.017	9	0.032	7	0.026	7	0.015
putative effectors	874	0.074	88	0.152	56	0.197	24	0.088	59	0.123
genes < 2 Kb Tes ^c	1723	0.147	112	0.193	83	0.292	79	0.288	64	0.133
orphan genes	1717	0.146	101	0.174	33	0.116	88	0.321	27	0.056
genes expressed at 4 dpi vs axenic ^d	559	0.048	55	0.095	0	NA	3	0.011	7	0.015
genes expressed at 13 dpi vs axenic ^d	886	0.075	121	0.209	2	0.007	25	0.091	9	0.019
genes expressed at 13 dpi vs 4 dpi ^d	729	0.062	105	0.181	12	0.042	44	0.161	21	0.044
genes up-regulated at 4 dpi vs. <i>in vitro</i> ^e	784	0.067	104	0.180	22	0.077	11	0.040	72	0.150
genes down-regulated at 4 dpi vs. <i>in vitro</i> ^f	1027	0.087	61	0.105	55	0.194	34	0.124	120	0.249
genes up-regulated at 13 dpi vs. 4 dpi ^e	1773	0.151	276	0.477	78	0.275	92	0.336	120	0.249
genes down-regulated at 13 dpi vs. 4 dpi ^f	798	0.068	48	0.083	61	0.215	15	0.055	113	0.235

Table 3. Continued

<i>Δkmt1/kmt6^h</i>				
	up-regulated		down-regulated	
	nb. in the genome	proportion	nb. in the genome	proportion
total number of genes	558		455	
H3K4me2 ^a	72	0.129	147	0.323
H3K9me3 ^a	8	0.014	10	0.022
H3K27me3 ^a	248	0.444	69	0.152
H3K9/K27me3 ^a	29	0.052	22	0.048
unknown ^b	420	0.753	157	0.345
CAZymes	7	0.013	10	0.022
putative effectors	65	0.116	62	0.136
genes < 2 Kb Tes ^c	138	0.247	125	0.275
orphan genes	166	0.297	50	0.110
genes expressed at 4 dpi vs axenic ^d	62	0.111	0	NA
genes expressed at 13 dpi vs axenic ^d	137	0.246	2	0.004
genes expressed at 13 dpi vs 4 dpi ^d	119	0.213	18	0.040
genes up-regulated at 4 dpi vs. <i>in vitro</i> ^e	94	0.168	25	0.055
genes down-regulated at 4 dpi vs. <i>in vitro</i> ^f	33	0.059	123	0.270
genes up-regulated at 13 dpi vs. 4 dpi ^e	229	0.410	117	0.257
genes down-regulated at 13 dpi vs. 4 dpi ^f	38	0.068	73	0.160

Table 3. Influence of *kmt1*, *kmt6* or *kmt1/kmt6* deletions on gene expression.

^aGenes located in a H3K4me2, H3K9me3, H3K27me3 or H3K9me3, H3K27me3 *in vitro*;

^b“unknown” genes are genes encoding predicted or hypothetical proteins;

^cGenes located up to 2 kb upstream or downstream to a transposable element sequence;

^dGenes specifically expressed at 4 or 13 days post infection (dpi) compared to axenic culture or 4 dpi when RPKM ≥ 2 at the time point and RPKM ≤ 2 during the axenic culture or at 4 dpi;

^eUp-regulated genes are genes with Log2 Fold Change (RPKM) ≥ 1 and FDR ≤ 0.001 in a given condition compared to the other;

^fDown-regulated genes are genes with Log2 Fold Change (RPKM) ≤ -1 and FDR ≤ 0.001 in a given condition compared to the other;

^hGenes deregulated in *KMT1*, *KMT6* or *KMT1/KMT6* deletions background, Log2 Fold Change (RPKM) ≤ -1 or ≥ 1 and FDR ≤ 0.001 .

Bold: the given domain contains significantly less genes of the given category compared to the rest of the genome; grey: the given domain is enriched for the category of genes compared to the rest of the genome.

Table 4. Expression of selected genes associated with heterochromatin *in vitro*, in a *kmt1*, *kmt6* and *kmt1/kmt6* deletion background.

							<i>in planta</i> expression ^d	relative expression vs. WT ^e		
ID	orphan ^a	TE ^b	SP ^a	H3K4me2 ^c	H3K9me3 ^c	H3K27me3 ^c	up 13 vs 4 dpi	$\Delta kmt1$	$\Delta kmt6$	$\Delta kmt1/kmt6$
Zt09_chr_2_00407	-	yes		-	yes		-	2	3	7
Zt09_chr_2_00483	yes	-	-	-	-	yes	-		3	2-8
Zt09_chr_3_00231	yes	yes	yes	-	-	yes	6	-	-	-
Zt09_chr_4_00279	-	yes	yes	-	yes		6	-	3-5	-
Zt09_chr_8_00723	yes	yes		-			3	2-5	200	20
Zt09_chr_9_00004	-	yes		-	yes	yes	3	6-16	65-90	~9
Zt09_chr_9_00005	-	yes	yes	-	yes	yes	8	-	40	~3
Zt09_chr_11_00525	-	yes		-	yes	yes	6	20-69	200-1000	140
Zt09_chr_13_00020	-	-	-	-	-	yes	10	1-4	28-36	6-9

^aGenes located up to 2 kb upstream or downstream to a transposable element sequence;

^bGenes located in a H3K4me2, H3K9me3, H3K27me3 or H3K9me3, H3K27me3 *in vitro*;

^cGenes significantly up-regulated 13 days post-infection (dpi) compared to 4 dpi;

^dGene expression relative to the WT strain and to the GAPDH encoding gene.

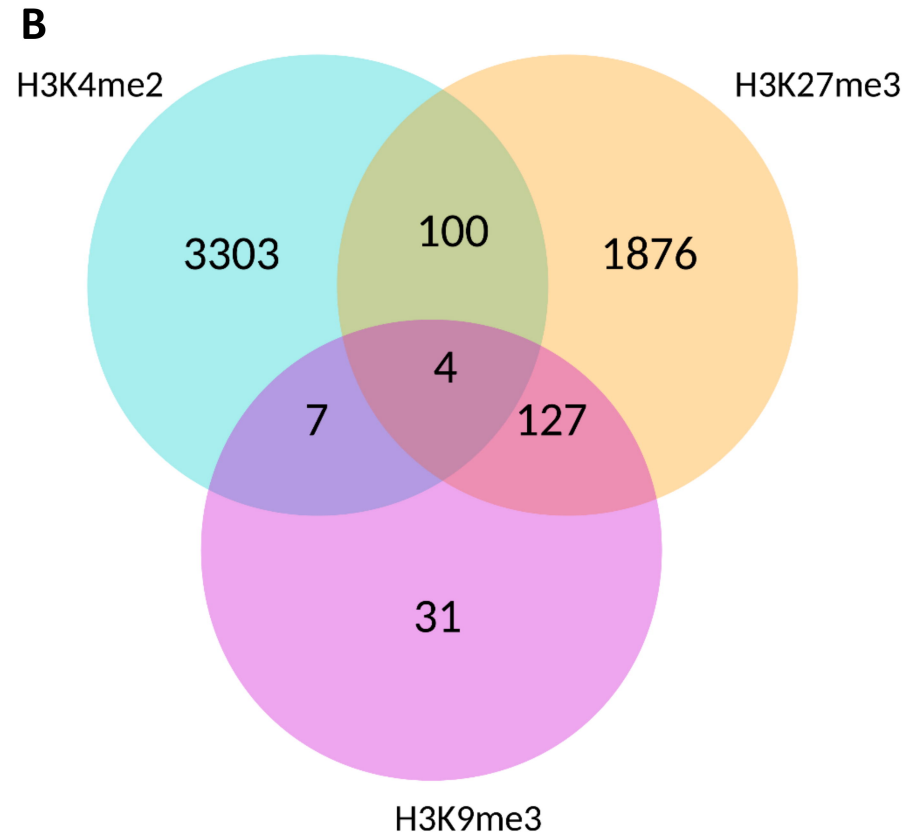
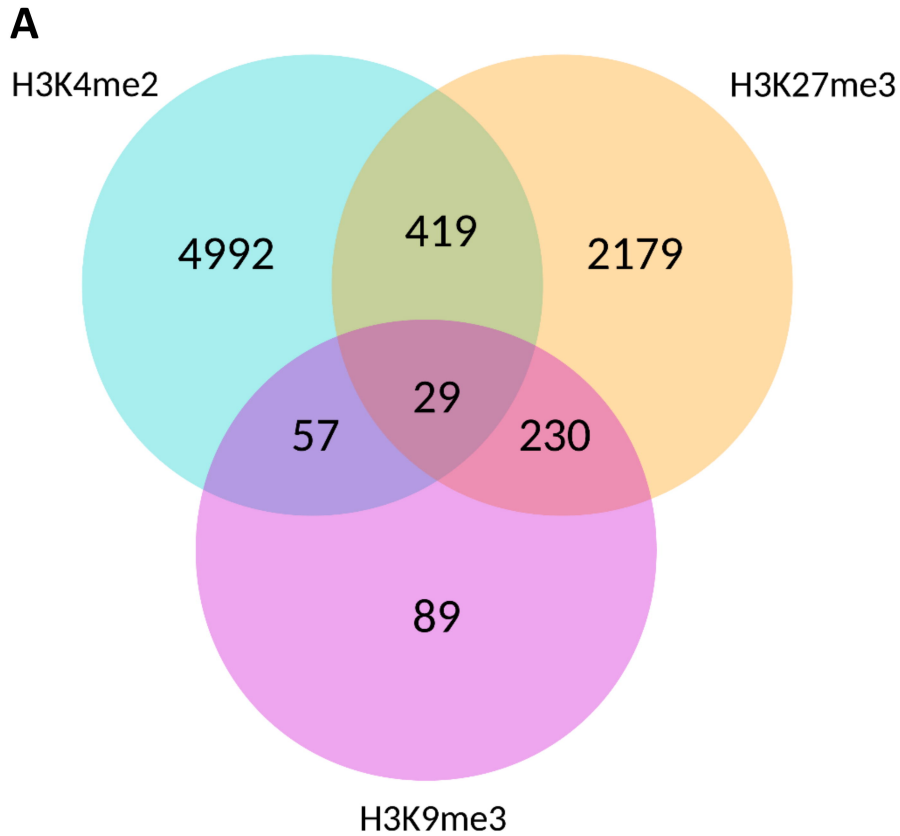


Figure 1. Euchromatic domains encompass more genes than heterochromatin domains *in vitro*. Venn diagrams showing genes associated with the different post-translational histone modifications assessed using ChIP-sequencing in axenic culture. A) Genes at least partially associated with any of the histone modification. B) Genes completely associated with any of the histone modifications.

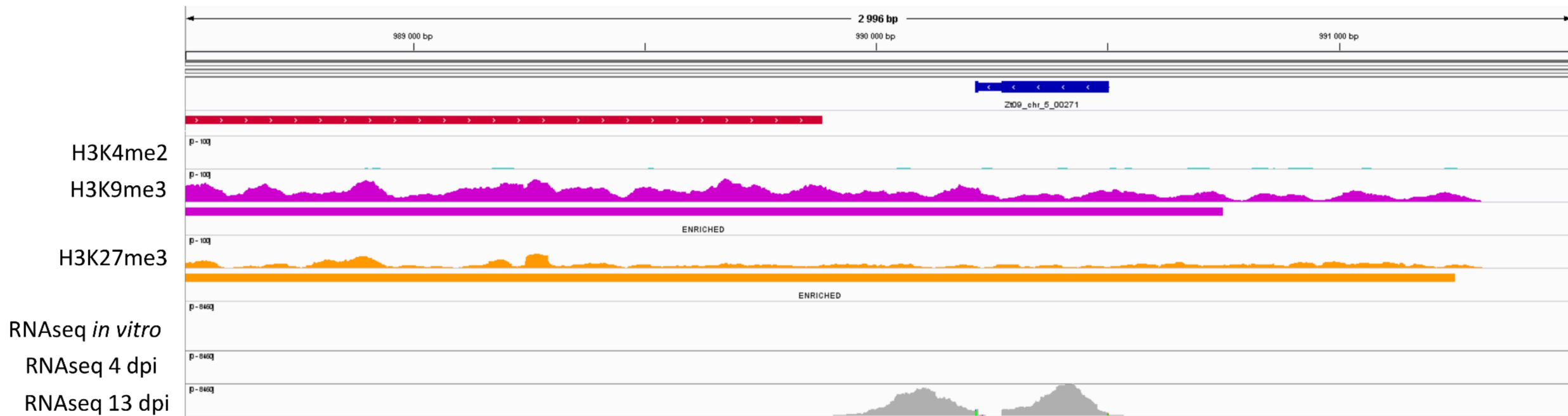


Figure 2. Heterochromatic domains are enriched with putative effector genes that are up-regulated during the switch towards necrotrophic growth on wheat. Example from a section of chromosome 5, harboring the putative effector gene ID *Zt09_chr_5_00271*. Regions enriched in different histone modifications were identified using ChIP-seq with antibodies against H3K4me2 (light blue), H3K9me3 (purple), H3K27me3 (orange) and compared to the location of genomic features (coding sequences, dark blue; transposable elements, red) and to reads obtained by RNA-seq in axenic culture, 4 days post-infection (dpi) and 13 dpi.

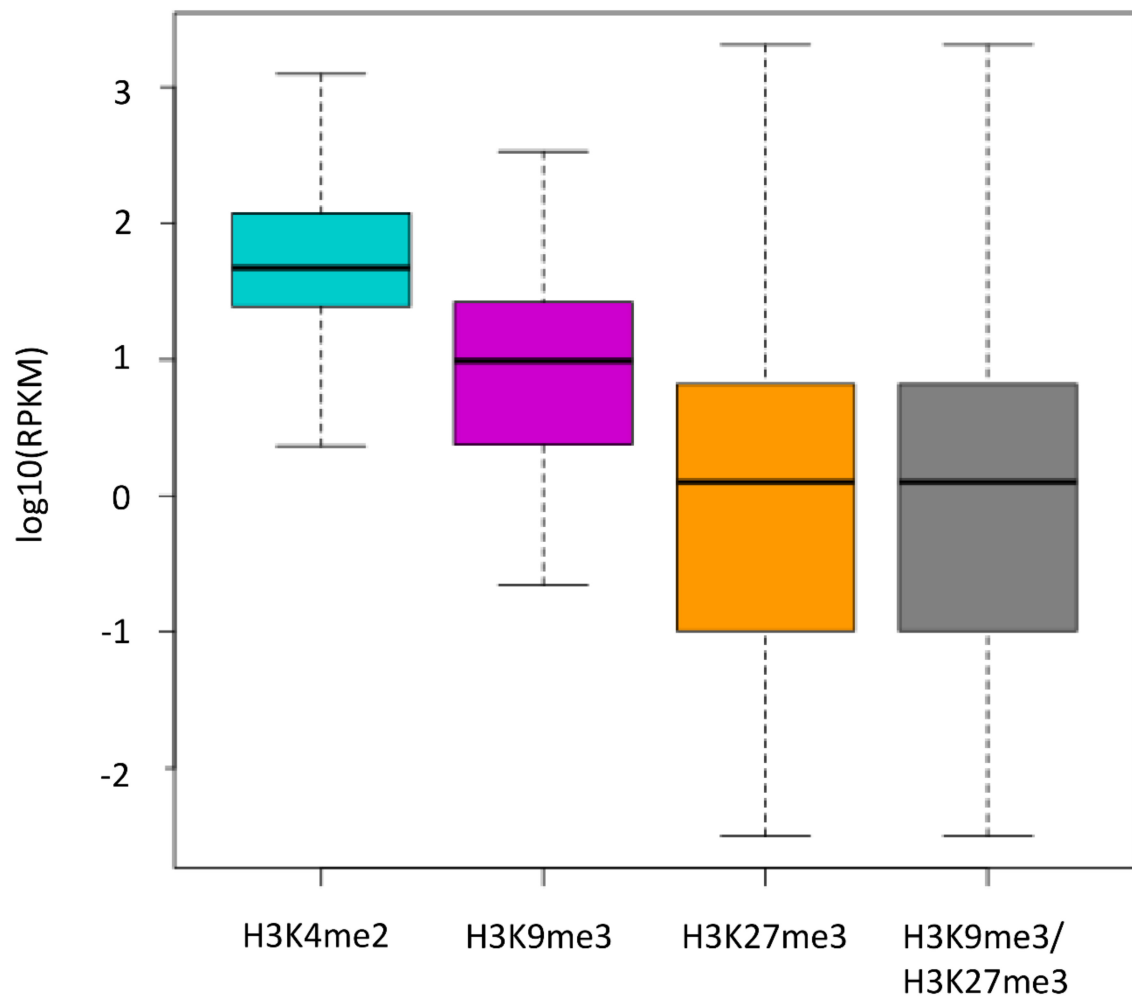


Figure 3. Genes associated with euchromatin are expressed while genes associated with heterochromatin are repressed. Expression assessed during axenic culture using RNA-sequencing. Boxplot of the $\log_{10}(\text{RPKM})$ of the genes totally associated with any of the histone modification.

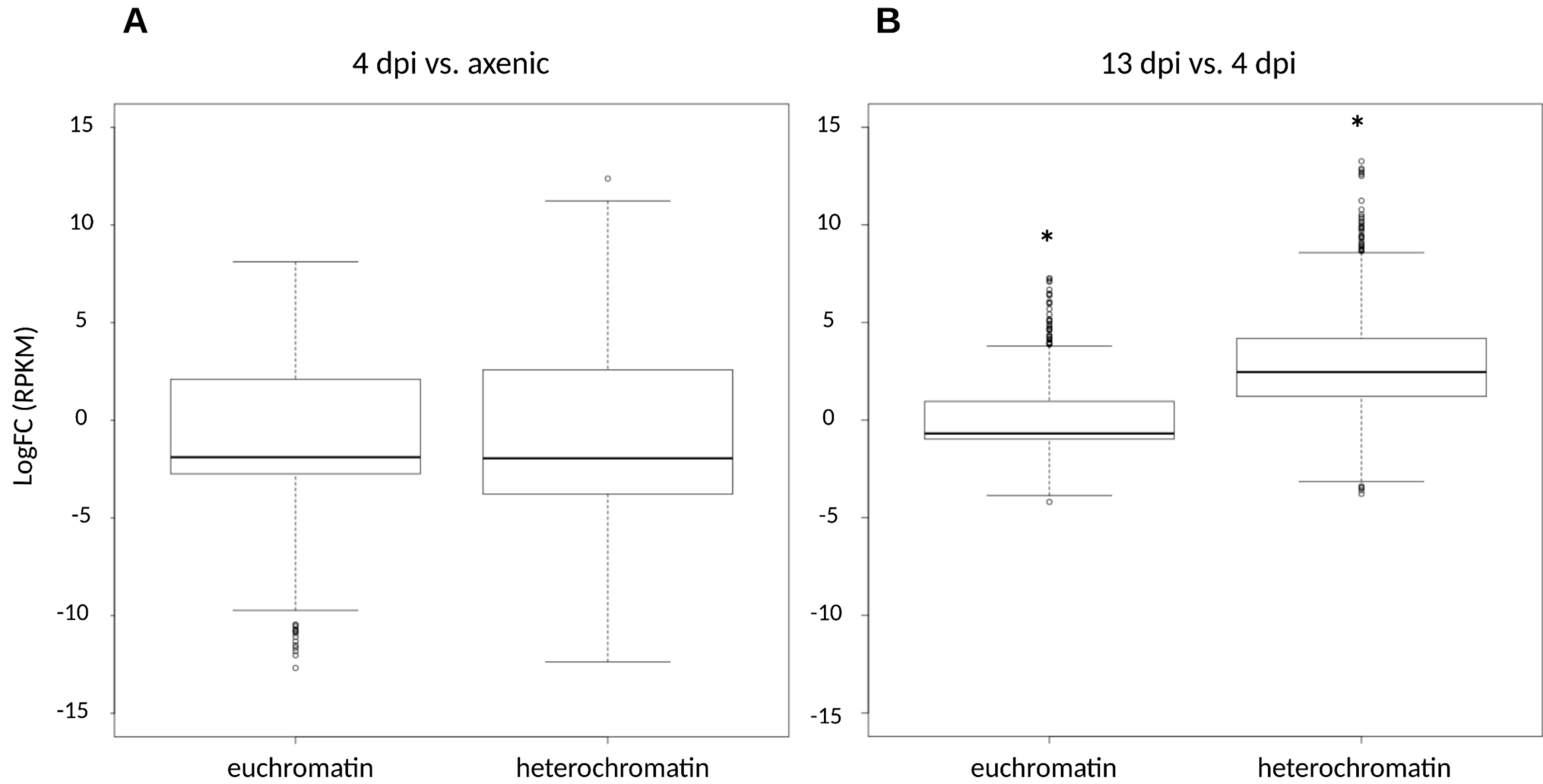


Figure 4. Genes associated with euchromatin and heterochromatin *in vitro* exhibit different expression profiles *in planta*. Boxplot of the $\log_{10}$ Fold Change (RPKM) of the genes located in a euchromatic domain *in vitro* (i.e. H3K4me2) or a heterochromatic domain *in vitro* (i.e. H3K9me3, H3K27me3 or H3K9me3/H3K27me3). Expression assessed during axenic culture and at 4 and 13 days post infection (dpi) using RNA-sequencing; genes differentially expressed at 4 dpi vs. axenic culture (A) or 13 dpi vs. 4 dpi (B). *: Wilcoxon-test, $P < 2.2 \cdot 10^{-16}$: comparison of heterochromatic-associated genes between 4 and 13 dpi.

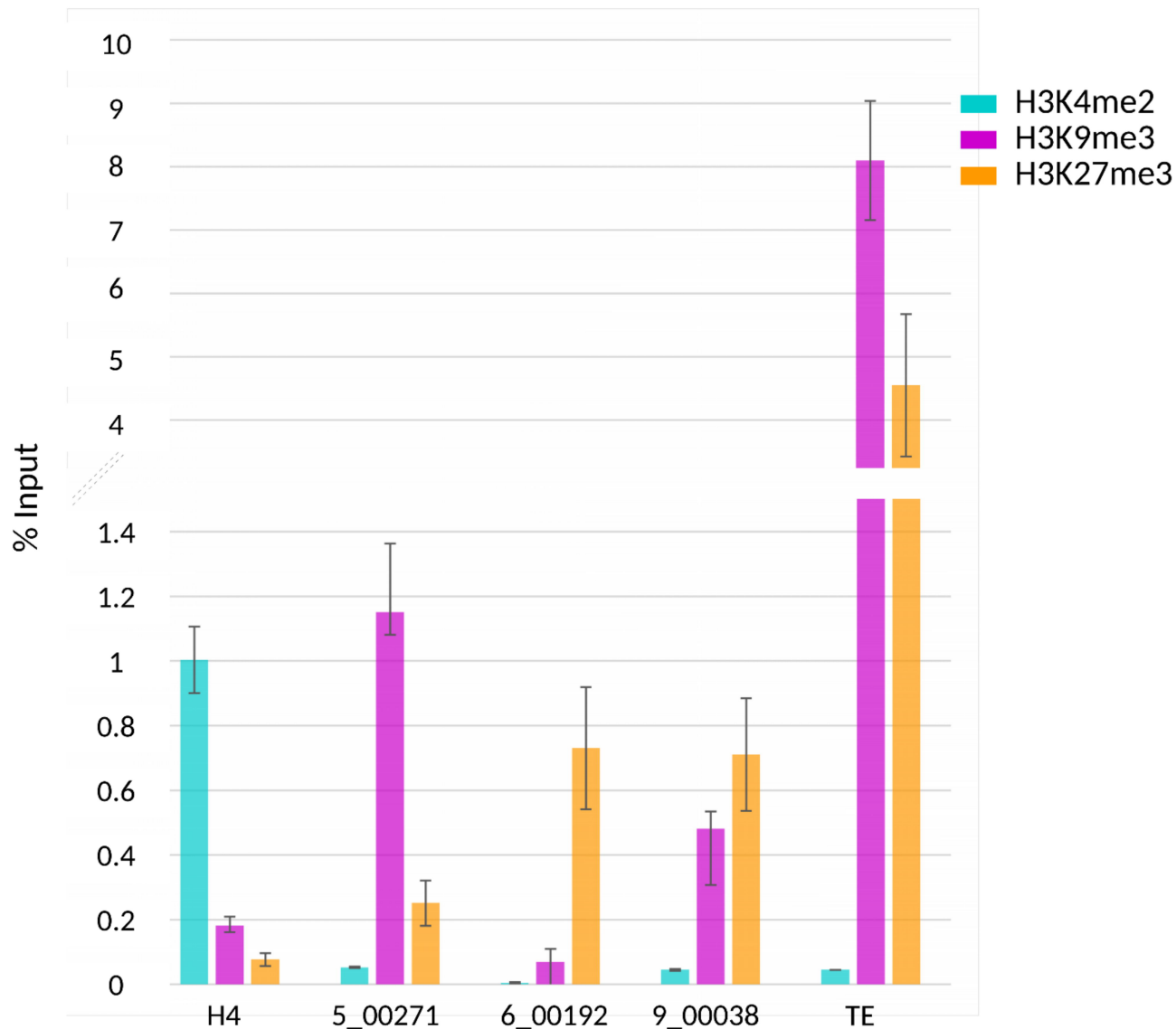


Figure 5. Putative effector genes are enriched in H3K9me3 and/or H3K27me3 *in vitro* while histone H4 is enriched in H3K4me2. Chromatin immunoprecipitation was performed during axenic culture to assess the levels of H3K4me2, H3K9me3 and H3K27me3 in the genomic sequence of four genes (H4, Histone H4: Zt09_chr_6_00256; 5_00271: Zt09_chr_5_00271; 6_00192: Zt09_chr_6_00192; 9_00038: Zt09_chr_9_00038) and a transposable element (DTH_element5_ZTIPO323).

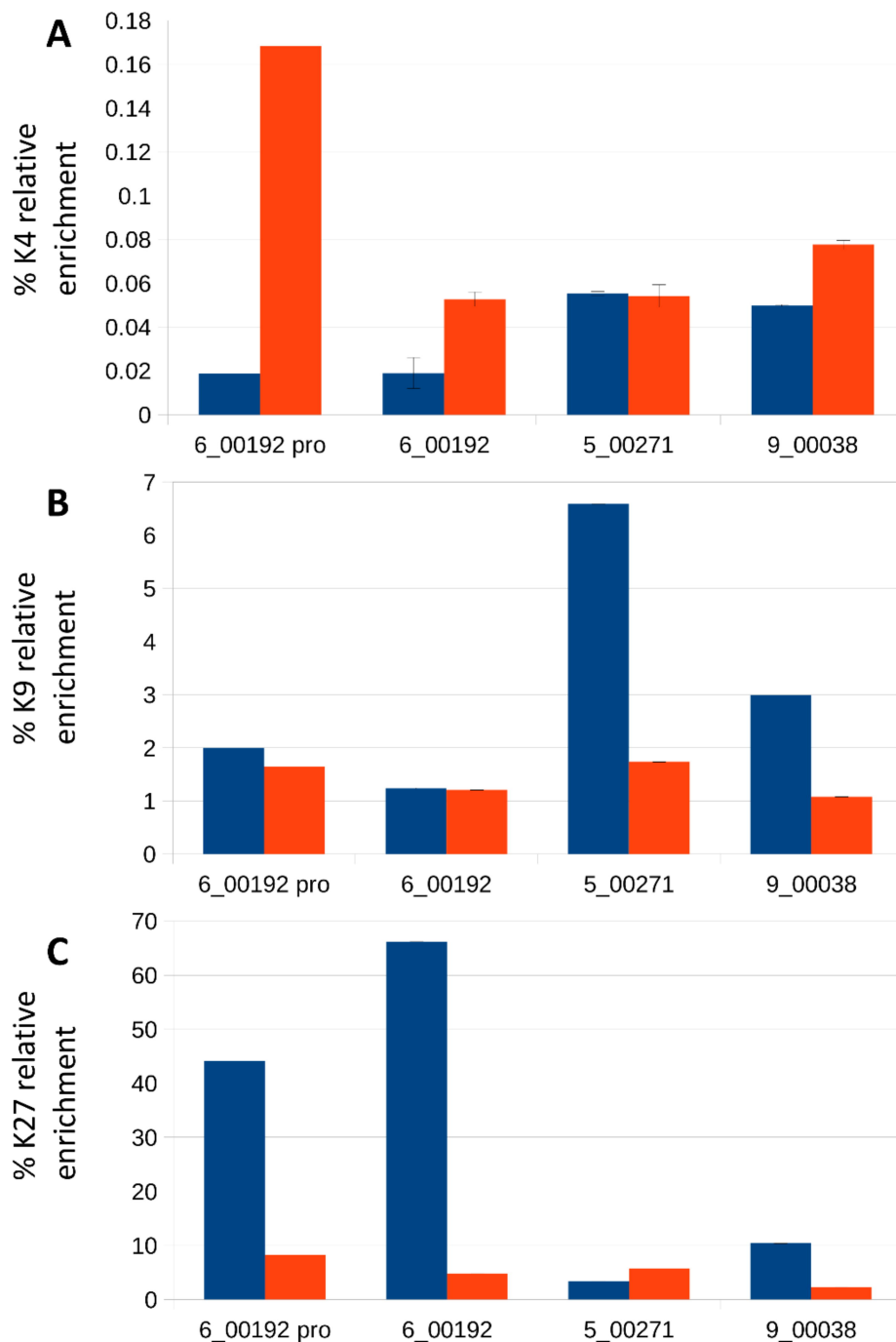


Figure 6. The up-regulation of three effector genes *in planta* is associated with a change of their histone modifications patterns. ChIP was performed at 13 days post infection of wheat leaves by *Zymoseptoria tritici* and relative enrichment of (A) H3K4me2, (B) H3K9me3 and (C) H3K27me3 was assessed using qPCR and compared during the axenic culture and at 13 dpi. 5_00271: Zt09_chr_5_00271; 6_00192: Zt09_chr_6_00192; 6_00192 pro: Zt09_chr_6_00192, promoter; 9_00038: Zt09_chr_9_00038; K4, K9 and K27: H3K4me2, H3K9me3 and H3K27me3 respectively. Blue: axenic culture; red: 13 dpi.