Local rather than global H3K27me3 dynamics associates with differential gene expression in *Verticillium dahliae*

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Running title: Global histone dynamics in *Verticillium dahliae*

Word count Abstract: 237

Word count Importance: 150

Word count text: 6.466

ABSTRACT

Differential growth conditions typically trigger global transcriptional responses in filamentous fungi. Such fungal responses to environmental cues involve epigenetic regulation, including chemical histone modifications. It has been proposed that conditionally expressed genes, such as those that encode secondary metabolites but also effectors in pathogenic species, are often associated with a specific histone modification, lysine27 methylation of H3 (H3K27me3). However, thus far no analyses on the global H3K27me3 profiles have been reported under differential growth conditions in order to assess if H3K27me3 dynamics governs differential transcriptional. Using ChIP- and RNA-sequencing data from the plant pathogenic fungus *Verticillium dahliae* grown on three *in vitro* cultivation media, we now show that a substantial number of the identified H3K27me3 domains globally display stable profiles among these growth conditions. However, we do observe local quantitative differences in H3K27me3 ChIP-seq signal that associate with differentially transcribed genes between media. Comparing the *in vitro* results to expression during plant infection suggests that *in planta* induced genes require chromatin remodelling to achieve expression. Overall, our results demonstrate that although some loci display H3K27me3 dynamics that can contribute to transcriptional variation, other loci do not show such dependence. Thus, we conclude that while H3K27me3 is required for transcriptional repression, it is not a conditionally responsive global regulator of differential transcription. We propose that the H3K27me3 domains that do not undergo dynamic methylation may contribute to transcription through other mechanisms or may serve additional genomic regulatory functions.

IMPORTANCE

In many organisms, including filamentous fungi, epigenetic mechanisms that involve chemical and physical modifications of DNA without changing the genetic sequence have been implicated in transcriptional responses upon developmental or environmental cues. In fungi, facultative heterochromatin that can de-condense to allow transcription in response to developmental changes or to environmental stimuli is characterized by tri-

methylation of lysine 27 on histone H3 (H3K27me3), and H3K27me3 has been implicated in transcriptional regulation although precise mechanisms and functions remain enigmatic. Based on ChIP- and RNA-sequencing data, we show for the soil-borne broad host range vascular wilt plant pathogenic fungus *Verticillium dahliae* that although some loci display H3K27me3 dynamics that can contribute to transcriptional variation, other loci do not show such dependence. Thus, although we recognize that H3K27me3 is required for transcriptional repression, we also conclude that this mark is not a conditionally responsive global regulator of differential transcription upon responses to environmental cues.

INTRODUCTION

The fungal kingdom comprises a plethora of species occupying an enormously diverse range of ecological niches (Schmit & Mueller, 2007). As environments are typically dynamic, including the effects of daily and yearly cycles, fungi continuously need to respond and adapt to survive (Gibson, 2008; Selvig & Alspaugh, 2011). To this end, fungi have evolved various mechanisms to monitor their environment and to transcriptionally respond to environmental cues (Bahn *et al.*, 2007). For instance, the yeast *Saccharomyces cerevisiae* senses cold stress by increased membrane rigidity, which leads to transcription of genes that, among others, encode cell damage-preventing proteins (Aguilera *et al.*, 2007). Furthermore, *S. cerevisiae* senses the quantitative availability of carbon and nitrogen sources in the environment to determine which developmental program maximises the potential for survival (Schneper *et al.*, 2004). In animal systems, epigenetic mechanisms (i.e. those affecting nuclear processes by modifying DNA characteristics without changing the genetic sequence) are implicated in transcriptional responses to changing environments (Heijmans *et al.*, 2008; Zannas *et al.*, 2015; Martin & Fry, 2018). Such epigenetic mechanisms have similarly been proposed to contribute to environmental response in filamentous fungi. For instance, the saprotrophic fungus *Neurospora crassa* phenotypically reacts to environmental stimuli, such as changes in temperature and pH, yet *N. crassa* mutants impaired in transcription-associated epigenetic mechanisms display reduced growth in response to these stimuli (Kronholm *et al.*, 2016). Similarly, the nectar-feeding yeast *Metschnikowia reukaufii* and the ubiquitous fungus *Aureobasidium pullulans* fail to properly respond to changing carbon sources when DNA methylation or histone acetylation are inhibited (Slepecky & Starmer, 2009; Herrera *et al.*, 2012). These results suggest that epigenetic mechanisms are important for transcriptional responses to changing environments in diverse fungi, but many questions remain regarding the precise mechanisms and function of epigenetic dynamics in fungi.

Epigenetic mechanisms, such as direct modifications of DNA and histone proteins or physical changes to the chromatin architecture, can influence transcription by

regulating DNA accessibility (Slotkin & Martienssen, 2007). Chromatin that is accessible and potentially active is termed euchromatin, while heterochromatin is condensed and often transcriptionally silent (Bannister & Kouzarides, 2011). However, heterochromatic regions are not always repressed. Heterochromatin is subcategorized into constitutive heterochromatin that remains condensed throughout the cell cycle, and facultative heterochromatin that can de-condense to allow transcription in response to developmental changes or to environmental stimuli (Trojer & Reinberg, 2007; Kim *et al.*, 2015; Wang *et al.*, 2016). In fungi, constitutive heterochromatin is often associated with repeat-rich genome regions and is typically characterized by tri-methylation of lysine 9 on histone H3 (H3K9me3), while facultative heterochromatin is characterized by tri-methylation of lysine 27 on histone H3 (H3K27me3) (Lewis *et al.*, 2009, 2010; Jamieson *et al.*, 2013; Freitag, 2017). Empirically, both H3K9me3 and H3K27me3 have been implicated in transcriptional regulation in various fungi (Reyes-Dominguez *et al.*, 2010, 2012; Connolly *et al.*, 2013; Wiemann *et al.*, 2013; Chujo & Scott, 2014; Soyer *et al.*, 2014). The majority of these studies rely on genetic perturbation of the enzymes that deposit methylation at H3K9 and H3K27, and the results consistently show that depletion of methylation at these lysine residues mainly results in transcriptional induction. However, as global depletion of a histone modification can result in pleiotropic effects, such as improper localization of other histone modifications or altered development (Tian & Chen, 2001; Fan *et al.*, 2017), it is difficult to infer transcriptional control mechanisms used for natural gene regulation from these genetic perturbation experiments. Therefore, additional research is needed to directly test the hypothesis that heterochromatin-associated histone modifications directly regulate transcription either through their dynamics, or their action to form or recruit transcriptional complexes (Galazka & Freitag, 2014; Seidl *et al.*, 2016; Ridenour *et al.*, 2020).

The filamentous fungus *Verticillium dahliae* is a soil-borne broad host-range plant pathogen that infects plants through the roots to invade the xylem vessels and cause vascular wilt disease (Fradin & Thomma, 2006). Genomic and transcriptomic studies have revealed that the *V. dahliae* genome harbours lineage-specific (LS) regions that are

variable between strains and enriched for genes that are *in planta* induced (Klosterman *et al.*, 2011; de Jonge *et al.*, 2013). These LS regions are generally considered genomic hotspots for evolutionary adaptation to plant hosts (Klosterman *et al.*, 2011; de Jonge *et al.*, 2013; Faino *et al.*, 2016; Shi-Kunne *et al.*, 2018; Depotter *et al.*, 2019; Cook *et al.*, 2020). Recently we explored the epigenome of *V. dahliae* and distinguished LS regions from the core genome based on particular chromatin signatures, including elevated levels of H3K27me3 accompanied with accessible DNA and active transcription (Cook *et al.*, 2020). Using a machine learning approach and supported by orthogonal analyses, we identified nearly twice as much LS DNA as previously considered, collectively referred to as adaptive genomic regions (Cook *et al.*, 2020). Given the elevated levels of H3K27me3 at adaptive genomic regions in *V. dahliae*, and previous reports that removal of H3K27me3 results in transcriptional induction (Connolly *et al.*, 2013; Chujo & Scott, 2014; Soyer *et al.*, 2014), we now tested if H3K27me3 dynamics are required for transcriptional activation of genes in adaptive genomic regions. Ideally, the involvement of H3K27me3 dynamics in differential gene expression of *V. dahliae* would be studied between *in vitro* and *in planta* growth, as adaptive genomic regions are enriched for *in planta* induced genes (de Jonge *et al.*, 2013; Cook *et al.*, 2020). However, *V. dahliae* displays a low pathogen-to-plant biomass during infection (Faino *et al.*, 2012), which impedes technical procedures to determine histone modification levels over the genome (Soyer *et al.*, 2015). Nevertheless, as H3K27me3 is generally reported to regulate transcription in response to environmental stimuli (Trojer & Reinberg, 2007; Wang *et al.*, 2016), we hypothesize that it may be involved in transcriptional regulation in *V. dahliae* during differential growth conditions *ex planta* as well. Here, we analyse RNA-seq, ChIP-seq and ATAC-seq data of *V. dahliae* cultured in various axenic growth media to understand if transcriptional dynamics require concomitant changes in the H3K27me3 modification status.

RESULTS

Genes induced between growth conditions are associated with H3K27me3

We sought to test if genes in adaptive genomic regions, which we recently reported to have distinct chromatin profiles (Cook *et al.*, 2020), are transcriptionally more responsive to differential *in vitro* and *in planta* growth conditions. We found that genes that are higher expressed in Czapec-Dox (CZA) or Murashige-Skoog (MS) media when compared with potato dextrose broth (PDB) are significantly enriched in adaptive genomic regions (two-sided two-proportions Z-test, $p < 0.01$) (Fig. 1A). More specifically, 125 (14.6%) out of 856, and 84 (11.8%) out of 711 genes that are higher expressed in CZA or MS than in PDB, respectively, locate in adaptive genomic regions, while these regions only comprise 998 (8.7%) of the total 11,426 genes in the genome (two-sided two-proportions Z-test, $p < 0.01$) (Fig. 1A). Interestingly, genes with greater transcriptional differences between the media (i.e. higher $\log_2$ -fold changes) appear to be increasingly more overrepresented in adaptive genomic regions than those with smaller or no differences (Fig. 1A).

We next compared expression during infection of *Arabidopsis thaliana* to cultivation in PDB. We found that genes that are higher expressed during *A. thaliana* colonization are also overrepresented in adaptive genomic regions, consistent with other host-infection experiments for *V. dahliae* (de Jonge *et al.*, 2013; Cook *et al.*, 2020). Similar to what we observed for *in vitro* growth conditions, genes with the greatest transcriptional dynamics are increasingly more overrepresented in adaptive genomic regions. That is, there are 145 (14.7%) out of 987 genes higher expressed *in planta* ($\log_2$ -fold change > 2) residing in adaptive genomic regions, while at $\log_2$ -fold change > 7 this increases to 26 (36.6%) out of 71 (Fig. 1A). These results show that genes higher expressed in other *in vitro* growth media compared to PDB, as well as genes that are higher expressed during plant infection compared to PDB, are overrepresented in adaptive genomic regions. Thus, these data suggest that genes that respond to environmental cues in general, rather than only genes that are induced *in planta*, are overrepresented at adaptive genomic regions.

Given the elevated levels of H3K27me3 at adaptive genomic regions in *V. dahliae* (Cook *et al.*, 2020), one potential explanation for the co-localization of genes that are differentially expressed *in vitro* and those that are differentially expressed *in planta* is

that these responses to different growth conditions share an adaptive origin that is epigenetically regulated by H3K27me3. However, given that H3K27me3 occurs not only in adaptive genomic regions in *V. dahliae*, but also throughout the core genome (Cook *et al.*, 2020), we sought to determine if H3K27me3 is associated with *in vitro* and *in planta* differentially expressed genes, not only in adaptive genomic regions, but also genome-wide. Thus, we first performed peak calling on H3K27me3-ChIP data of *V. dahliae* cultivated for 6 days in PDB and obtained 304 H3K27me3 domains that cover approximately 10.5 Mb (28.9%) of the genome (Fig. S1), comprising 3,280 (28.7%) genes and overlapping 87.4% of adaptive genomic regions. Interestingly, the observed overrepresentation of differentially expressed genes in the generally H3K27me3-marked adaptive genomic regions extends to H3K27me3 domains throughout the genome, showing that H3K27me3 is genome-wide associated with differentially expressed genes (Fig. 1B). Specifically, when assessing what fraction of higher expressed genes in CZA, MS or Arabidopsis when compared with PDB locate in an H3K27me3 domain, we found that 421 (49.2%) out of 856, 271 (38.1%) out of 711, and 453 (45.9%) out of 987 locate in H3K27me3 domains, respectively (Fig. 1B, log₂-fold change >2), which is considerably more than the 28.7% genome-wide average. Additionally, similar to our observations for adaptive genomic regions, we also observed that genes displaying higher log₂-fold changes were increasingly overrepresented in H3K27me3 domains. Here we found that 41 out of 51 (80.4%), 4 out of 7 (57.1%), and 54 out of 71 (76.0%) genes higher expressed with a log₂-fold change >7 in CZA, MS and Arabidopsis, respectively, located in H3K27me3 domains (Fig. 1B). Collectively, these data reveal a strong association between differential gene expression and the presence of H3K27me3 domains, both for genes that are differentially expressed between *in vitro* growth conditions, as well as for genes that are higher expressed *in planta* than *in vitro*. As we observed that the association with differentially expressed genes is not limited to adaptive genomic regions, but is a feature for H3K27me3 domains in general, we infer that H3K27me3 may be involved in regulating transcriptional changes in response to environmental cues.

Figure 1. Genes that are higher expressed in CZA, MS and *in planta* than in PDB are overrepresented in adaptive genomic regions and in H3K27me3 domains. Fractions of total genes and genes that are higher expressed than in PDB that locate in (A) adaptive genomic regions and (B) H3K27me3-enriched regions. Increased expression is determined between six days of cultivation *in vitro* of *V. dahliae* in potato dextrose broth (PDB) and 6 days of cultivation in Czapec-Dox (CZA) and half-strength Murashige Skoog medium (MS), and infection of *Arabidopsis thaliana* (21 dpi) (*in planta*). Fractions of higher expressed genes that reside in adaptive genomic regions or H3K27me3-enriched regions are reported for log2-fold changes >1, 2, 3, 4, 5, 6 and 7, respectively. H3K27me3-enrichment was determined for *V. dahliae* cultivation during six days in PDB. Black numbers above the bars indicate numbers of genes per gene set. White numbers at bottom of the bars indicate the numbers of genes that locate in adaptive genomic or H3K27me3-domain per gene set. Significance of fraction differences was calculated for each fraction of higher expressed genes in adaptive genomic regions when compared with the fraction of total genes in adaptive genomic regions, by the two-sided two-proportions Z-test (*: $p \leq 0.05$).

Chromatin features correlate with gene expression levels

To generally assess how chromatin impacts transcriptional activity in *V. dahliae*, we determined the occurrence of the heterochromatic histone mark H3K9me3, the euchromatic histone mark H3K4me2, and chromatin accessibility determined by assay for transposase accessible chromatin followed by sequencing (ATAC-seq) (Buenrostro *et al.*, 2015). Grouping the *V. dahliae* genes into five expression quintiles, from quintile 1 containing the highest 20% expressed genes to quintile 5 with the 20% lowest expressed

genes, we are able to integrate histone modification profiles and transcriptional activity. Expressed genes (quintiles 1-4) displayed low H3K4me2 coverage upstream of the transcription start site (TSS), followed by a steep increase of coverage over the start of the gene that decreases over the gene body (Fig. 2B). The strength of the association corresponds to the level of transcription, also within quintiles (Fig. S2). The low H3K4me2 coverage near the TSS coincides with increased chromatin accessibility, where higher expressed quintiles have more open DNA (Fig. 2B, Fig. S2). There is little evidence of H3K9me3 over gene bodies and *cis*-regulatory regions (Fig. 2B, Fig. S2), which corroborates that H3K9me3 marks TEs in constitutive heterochromatic regions such as the centromeres (Lewis *et al.*, 2009; Schotanus *et al.*, 2015; Cook *et al.*, 2020; Seidl *et al.*, 2020). During cultivation in PDB, H3K27me3 is mainly present on genes that are lowly or not expressed (quintile 4 and 5) (Fig. 2B, Fig. S2). Collectively, this empirical description of chromatin and transcriptional activity is consistent with reports for other fungi (Reyes-Dominguez *et al.*, 2010; Connolly *et al.*, 2013; Möller *et al.*, 2019).

To further analyse the association between H3K27me3 and gene expression, we generated expression quintiles for the genes located in H3K27me3 domains during PDB growth (3,280 genes). Interestingly, we found that genes in this group with higher expression values (e.g. quintile 1 and 2) had higher H3K4me2 over the gene body, more accessible promoters, and lower H3K27me3 values (Fig. 2C). This suggests that genes in H3K27me3 domains are not uniformly repressed or heterochromatic, and there appeared to be a quantitative, rather than qualitative association between H3K27me3 association and gene activity. Genes with a lower association to H3K27me3 may represent loci that are not in a stable state under the tested conditions, where some cells have a more euchromatic profile and other have a more heterochromatic profile. While we cannot infer these details from the current data, it is clear that genes with lower expression in PDB are generally marked with H3K27me3, have less H3K4me3 and have less accessible DNA in the region of transcription initiation (i.e. their promoter).

Figure 2. Lowly and non-expressed genes associate with H3K27me3. A) Whole genome distribution of the euchromatin-associated histone modification H3K4me2, the constitutive heterochromatin-associated histone modification H3K9me3, the facultative heterochromatin-associated histone modification H3K27me3 and chromatin accessibility as determined by ATAC-seq, based on chromosome 5 as an example. Genes are indicated in light blue, transposons are indicated in red, centromere is indicated in dark blue and adaptive genomic regions are indicated in yellow. B,C) Relative coverage of chromatin accessibility and the histone marks H3K4me2, H3K9me3, H3K27me3 over gene bodies (between transcription start site (TSS) and transcription end site (TES)) ± 800 bp of flanking sequence, grouped into quintiles based on gene expression levels upon cultivation for six days potato dextrose broth (PDB), for (B) all genes, and for (C) all H3K27me3-enriched genes.

Genetic perturbation of H3K27me3 induces transcription of many genes that are differentially expressed *in vitro* and *in planta*

To further characterize the influence of H3K27me3 on gene expression in *V. dahliae*, we deleted the histone methyltransferase component of the Polycomb Repressive Complex 2 (PRC2), termed *Set7* ($\Delta Set7$), leading to loss of H3K27me3 (Fig. S3). As H3K27me3 is

generally associated with facultative heterochromatin, we anticipated that the loss of H3K27me3 would mainly lead to induction of genes that were located in H3K27me3 domains in wild-type *V. dahliae*. Out of the 839 genes that are induced in $\Delta Set7$ ($\log_2$ -fold change >2 , $p<0.05$), 615 (73.3%) locate in H3K27me3 domains, which is significantly higher than expected given that only 28.7% of genes locate in H3K27me3 domains (Fisher's exact test, $p<0.00001$). In contrast, we find that 214 (28.0%) of 765 repressed genes in $\Delta Set7$ are in H3K27me3 domains (no association, Fisher's exact test, $p=0.78$) (Fig. 3A,B). Additionally, when comparing $\log_2$ -fold changes in expression between $\Delta Set7$ and the wild type strain, we observed that genes located in a H3K27me3 domain in wild type are more significantly induced in $\Delta Set7$ than genes not locating in H3K27me3 domains (Two-sample Student's T-test, $p<0.001$) (Fig. S4). These findings suggest that H3K27me3 represses transcription at marked regions.

We next sought to integrate the repressive transcriptional effects of H3K27me3 inferred from $\Delta Set7$ RNA-seq with the differential transcription observed between growth conditions. For this, we compared genes induced in $\Delta Set7$ versus wild-type PDB growth with genes higher expressed in CZA, MS or *in planta* versus PDB growth. There is a significant overrepresentation of genes induced in $\Delta Set7$ (839), and those induced in CZA (254 genes, 30.3% overlap, Fisher's exact test $p<0.00001$), MS (197 genes, 23.5% overlap, Fisher's exact test $p<0.00001$) and *in planta* (208 genes, 24.8% overlap, Fisher's exact test $p<0.00001$). These associations are higher than the background levels of genes induced under CZA, MS or *in planta*, which is 7.5%, 6.2%, and 8.6% of genes, respectively (Fig. 3C-E). Together, these data show that H3K27me3 is required for transcriptional silencing of mainly H3K27me3 associated genes, and that many of these genes are also differentially expressed between the tested growth conditions. These results support the hypothesis that transcriptional dynamics between the tested growth conditions are regulated, at least partly, by levels of H3K27me3.

Figure 3. Genetic perturbation of H3K27me3 results in induction of genes that are transcriptionally regulated in different growth conditions. A) Fractions of induced (log2 fold change >1, p<0.05) and repressed (log2 fold change <-1, p<0.05) genes and genes that are not differentially expressed between wild-type and $\Delta Set7$ that (B) locate in H3K27me3 domains and (C-E) are higher expressed in CZA, MS and *in planta* than in PDB.

H3K27me3 domains are globally stable between *in vitro* growth conditions

Under the hypothesis that H3K27me3 dynamics contribute to differential gene expression, we predict that H3K27me3 ChIP-seq domains will change when *V. dahliae* is grown under different conditions. In addition, the observed changes in H3K27me3 domains will be associated with transcriptional differences of the underlying genes. To test this hypothesis, we performed additional H3K27me3 ChIP-seq for *V. dahliae* cultured in MS and CZA, and identified H3K27me3 domains from replicate H3K27me3 ChIP-seq in each of the three growth media. We identified a total of 1,631 genes that were always present in H3K27me3 domains, regardless of the *in vitro* growth media (Fig. 4A). Not surprisingly, the 1,631 genes present in stable H3K27me3 domains were significantly lower expressed when compared with non-marked genes in all three growth conditions (Fig. 4B). To assess if changes in H3K27me3 domains between the media were associated with changes in transcription, we compared domains and gene expression between media. We first analysed RNA-seq output for genes present in H3K27me3

domains in two media but not in an H3K27me3 domain in the third medium. For example, we identified 703 genes that were in a H3K27me3 domain in PDB and in MS but not in CZA (Fig. 4A), and found that these genes were more highly expressed during CZA growth when compared with growth in PDB or in MS (Fig. 4C). These result corroborated the association between the lack of H3K27me3 in CZA and increased transcription of the underlying genes. However, genes present in shared H3K27me3 domains for MS and CZA growth (229), or CZA and PDB growth (44) did not display statistically significant transcriptional differences between the growth conditions where the genes lack H3K27me3 domains (Fig. 4A, C). Analysing H3K27me3 domains unique to a media, we found that MS growth had the greatest number and proportion of unique genes locating in H3K27me3 domains (18.3%), followed by 12.4% unique to CZA growth, and 4.7% unique to PDB growth (Fig. 4A). The genes uniquely marked in PDB had a non-significant, but lower median expression value when compared with the other two media. Genes uniquely located in MS H3K27me3 domains were significantly lower expressed when compared with growth in CZA, but not with growth in PDB (Fig. 4D). The genes uniquely present in CZA H3K27me3 domains did not display transcriptional differences when *V. dahliae* was grown in the other two media. Overall, these results show that H3K27me3 contributes to transcriptional repression, but there is not a simple global association between H3K27me3 presence/absence dynamics and transcriptional output. We observed clear examples where the loss of H3K27me3 in one medium is associated with increased transcription in that medium, but this is not ubiquitous. We note that the majority of genes located in H3K27me3 domains were common to all three growth conditions, accounting for 75%, 65.4% and 52% of the identified genes in H3K27me3 domains from CZA, PDB, and MS growth, respectively, indicating that the qualitative presence of H3K27me3 domains is globally stable.

Figure 4. Differential H3K27 methylation partially explains differential expression. A) The number of genes located in H3K27me3 domains for *V. dahliae* cultivated for 6 days in Czapec-Dox (CZA), half-strength Murashige Skoog medium (MS) and Potato dextrose broth (PDB). B) Transcriptional values from RNA-seq, represented as TPM values in each growth medium. The genes were grouped between those consistently localized in H3K27me3 domains (1631 genes) and genes not present in H3K27me3 domains. C,D) Transcription TPM values in each growth medium for genes in H3K27me3 domains found in C) two or D) one of three tested *in vitro* growth media. Median values of violin plots are indicated with diamonds. Significant difference of TPM values between growth media are determined with the One-Sample Wilcoxon Signed Rank Test (*: $p \leq 0.05$).

Local quantitative differences in H3K27me3 levels are associated with transcriptional differences

Our analysis on H3K27me3 presence/absence dynamics did not account for potential quantitative differences between growth media. Whole chromosome plots of H3K27me3 domains identified between media reflect their generally stable presence (Fig. S5A), but the quantitative level of ChIP-seq signal appeared to vary at some loci within called domains (Fig. S5B). To address this, we examined quantitative differences between H3K27me3 ChIP-seq signal and transcriptional output between pairs of growth conditions. Genes were grouped based on differential gene expression between media,

where genes higher expressed in medium A were one group and genes higher expressed in medium B another group. The H3K27me3 ChIP-seq levels were compared between the groups for the ChIP conducted in both the transcriptionally permissive and non-permissive growth conditions. Comparing results for PDB versus MS, we see that genes higher expressed in MS have a significantly lower H3K27me3 signal from MS-ChIP versus the same genes from PDB-ChIP (Fig. 5A). For genes more highly expressed in PDB, there is a higher, but not significantly different, H3K27me3 ChIP-seq level when grown in MS when compared with PDB (Fig. 5A). These results support the idea that when a gene is transcriptionally active in one media, the H3K27me3 levels are lower than H3K27me3 levels under less transcriptionally permissive growth. Integrating the transcriptional and ChIP fold-changes for all genes between the media, we see that this association is true for some, but not all genes that undergo transcriptional changes between the media (Fig. 5B). Globally, the distribution of H3K27me3 fold-changes per gene between media shows that genes higher expressed in PDB have a significantly higher H3K27me3 fold-change (i.e. less H3K27me3 in PDB growth), while MS higher expressed genes have a significantly lower H3K27me3 fold-change (i.e. less H3K27me3 in MS growth) (Fig. 5B).

Results for PDB versus CZA growth showed that genes higher expressed in CZA have higher H3K27me3 levels in PDB, consistent with the expected association (Fig. 5C). For genes higher expressed in PDB however, we observed a higher H3K27me3 level in PDB. Globally, the data indicated a lower H3K27me3 ChIP-signal for samples grown in CZA (Fig. 5D, data shifted to left on x-axis). The integrated results indicated that genes higher expressed in CZA had less H3K27me3 in CZA, but the PDB higher expressed genes also had lower H3K27me3 in CZA. This difference, the overall lower H3K27me3 levels in CZA, may represent a biologically relevant difference between the growth media, but may also reflect a technical bias encountered for ChIP using CZA grown material. It is not possible to fully resolve these two possibilities with our current data.

Results for CZA versus MS growth showed that genes higher expressed in MS had statistically similar levels of H3K27me3 ChIP-signal between the two media (Fig. 5E). For genes higher expressed in CZA, there was significantly higher H3K27me3 ChIP-signal

during MS growth (Fig. 5E). The genome-wide integrated analysis, combining fold-change between CZA and MS for transcription and H3K27me3 ChIP, showed that genes more highly expressed in CZA indeed have significantly higher levels of H3K27me3 when grown in MS (Fig. 5F). We again see the effect of the lower H3K27me3 ChIP-signal from CZA-grown material, where the global fold-change values indicate higher levels of H3M27me3 in MS-grown material overall (Fig. 5F). However, there is a clear group of genes that are more highly expressed in MS medium that have an H3K27me3 fold-change indicating a shift toward higher levels in CZA. This can be seen by the significantly different marginal density plot for H3K27me3 fold-change, where the genes higher expressed in MS have a significantly greater change towards higher H3K27me3 levels in CZA (Fig. 5F). Overall, the results show that in most comparisons, there is an association between quantitative transcriptional levels and H3K27me3 signal, where genes more highly expressed in a transcriptionally permissive media have less H3K27me3 when compared to H3K27me3 levels in the repressive media. These results are consistent with the observations of the qualitative H3K27me3 presence/absence comparisons and suggests that H3K27me3 levels are generally stable, but can vary for some genes in a quantitative manner to impact their transcription.

Figure 5. Differentially expressed genes associate with local changes in H3K27me3 coverage. Pairwise comparisons of differentially expressed genes for *V. dahliae* cultivated for 6 days in A,B) half-strength Murashige Skoog medium (MS) and Potato dextrose broth (PDB), C,D) Czapec-Dox (CZA) and PDB, and E,F) MS and CZA. A,C,E) H3K27me3 coverage over differentially expressed genes in each corresponding growth medium. Median values of violin plots are indicated with diamonds. B,D,F) per gene comparison of log₂-fold change in transcription and genic H3K27me3 coverage. Black horizontal lines indicate TPM log₂-fold change of -2 and 2, black vertical lines indicate H3K27me3 log₂-fold change of -1 and 1. Significant difference of TPM values between growth media are determined with the One-Sample Wilcoxon Signed Rank Test and indicated by letters or by asterisks (*: $p \leq 0.05$).

Genes induced *in planta* are largely H3K27me3 associated across all tested growth conditions

The presented analysis address histone modification and transcriptional dynamics between axenic growth medium. A significant question is how do H3K27me3 levels compare between more environmentally drastic growth conditions, such as *in vitro* versus *in planta* growth. As we have thus far been unable to perform ChIP on *V. dahliae* during host colonization due to a low pathogen-to-plant biomass, we assessed the *in vitro* chromatin profiles for genes that are significantly induced during infection. First, we grouped genes based on differential expression between PDB and *in planta* growth and analysed the PDB grown chromatin profiles. We saw that genes higher expressed *in planta* have significantly more H3K27me3 during PDB growth when compared with the genes more highly expressed in PDB (Fig. 6A). These *in planta*-induced genes lacked H3K4me2 and the DNA is less accessible during PDB growth, while genes highly expressed in PDB are open and in active euchromatin (Fig. 6A). This pattern of *in planta*-induced genes being highly H3K27me3-associated is also observed when analysed relative to growth in MS and CZA (Fig. 6B,C).

To understand what genes are driving these differences, we investigated whether *in planta*-induced genes that locate in H3K27me3 domains are overrepresented for genes potentially involved in infection. There were approximately 1,000 genes more highly expressed *in planta* when compared with any of the three media (Fig. 6D). We found that, depending on the medium, from 36.5% to 53.4% of the *in planta*-induced genes were located in H3K27me3 domains (Fig. 6D). We observed that the *in planta*-induced genes within H3K27me3 domains have a higher, yet not statistically significant, fraction of genes encoding secreted proteins or putative effectors when compared to all *in planta* induced genes. Specifically, for genes in H3K27me3 domains that were *in planta*-induced compared to PDB growth (376 genes total), 16.0% had a secretion signal and 5.1% were predicted effectors. This is compared to all *in planta* induced genes relative to PDB (987 genes total), where 12.3% had a secretion signal and 3.2% were predicted effectors. For CZA, 17.0% of the genes in H3K27me3 domains that were *in planta*-induced (418 genes

total) had a secretion signal and 5.7% were predicted effectors. This is compared to all *in planta* induced genes relative to CZA (1,144 genes total), where 13.4% had a secretion signal and 3.8% were predicted effectors. The results were similar for comparison to MS grown samples. Thus, we consistently observed that *in planta*-induced genes in H3K27me3 domains have a slightly higher fraction of genes that are potentially involved in infection. Collectively, these results indicate that genes required for infection associate with H3K27me3 and that chromatin dynamics are likely needed during *in planta* infection to increase DNA accessibility concomitant with *in planta* transcription compared to the measured state in PDB. Whether or not this activation is accompanied by loss or dynamics of H3K27me3 levels will need to be directly assessed *in planta* in the future.

Figure 6. *In planta*-induced genes are H3K27me3-associated under transcriptionally repressive conditions. Average coverage of chromatin features over genes differentially expressed between *in planta* and A) 6 days of cultivation in Potato dextrose broth (PDB), B) half strength Murashige Skoog medium (MS), and C) Czapec-Dox medium (CZA). D) overlap of genes that are higher expressed *in planta* than in PDB, MS or CZA with genes located in an H3K27me3 domain in the corresponding growth medium. Significant differences of H3K27me3 coverage are determined with the One-Sample Wilcoxon Signed Rank Test (*: $p \leq 0.05$).

DISCUSSION

Chemical histone modifications play an essential role in transcriptional regulation, but questions remain regarding their mechanism, especially in filamentous fungi. In our study we show that many differentially transcribed genes between *in vitro* and *in planta* growth are located in previously defined adaptive genomic regions (Cook *et al.*, 2020). We report that a majority of the genes identified as being differentially transcribed between the tested conditions reside in H3K27me3 domains. This mark only occupies 28.7% of the genes in the genome, but the rate of H3K27me3 association significantly increases for differentially expressed genes by 35% to 70%.

Previous reports propose that H3K27me3 represses transcription of secondary metabolite clusters in *Fusarium spp.* and *Epichloe festucae*, and effectors in *Zymoseptoria tritici* and *Magnaporthe oryzae* (Connolly *et al.*, 2013; Chujo & Scott, 2014; Studt *et al.*, 2016; Möller *et al.*, 2019; Zhang *et al.*, 2021). However, these reports have mainly relied on genomic association with H3K27me3, genetic perturbation altering H3K27me3 deposition, and the analysis of only a few genes under natural conditions. Given these previous findings, our hypothesis was that H3K27me3 levels are dynamic during growth in different conditions and contribute to differential transcription. We directly addressed this using genome-wide H3K27me3 profiling and RNA-seq under different *in vitro* growth conditions. Consistent with reports in other fungi, we see that H3K27me3 is associated with transcriptional repression, and that deleting *Set7*, encoding the histone methyltransferase that is responsible for H3K27me3, led to induction of many genes normally present in H3K27me3 domains. Importantly, our direct observations of H3K27me3 levels and transcriptional output from three *in vitro* media showed that H3K27me3 domains are generally stable globally. We see that 50% to 75% of the identified H3K27me3 domains, depending on the medium analyzed, did not change between the tested conditions. Despite many H3K27me3 domains not undergoing presence/absence dynamics, numerous genes in these domains displayed differential transcription between the tested conditions, indicating that complete loss of H3K27me3 is not strictly required for transcriptional induction.

Even though our data suggest that qualitative H3K27me3 changes are not sufficient to explain differential transcription under natural conditions alone, we do provide empirical evidence that quantitative H3K27me3 dynamics are associated with differentially expressed genes in *V. dahliae* grown under different *in vitro* conditions. These results show that some genes grown in transcriptionally permissive media have a lower H3K27me3 ChIP-signal when compared with growth in a transcriptionally repressive media. We interpret these results as evidence for local, not global, contribution of H3K27me3 quantitative dynamics to transcriptional regulation for these genes. These results are consistent with data from *M. oryzae*, in which direct *in planta* H3K27me3 ChIP-qPCR showed that some, but not all, analyzed regions displayed differential H3K27me3 levels between *in planta* and *in vitro* conditions (Zhang *et al.*, 2021). We have not been able to directly assess histone modifications for *V. dahliae* during plant infection, but we did analyze the *in vitro* growth H3K27me3 status of *in planta*-induced genes. The identified *in planta*-induced genes have significantly higher levels of H3K27me3 and the DNA is substantially less accessible compared to genes highly expressed during *in vitro* growth. We conclude that dynamics for H3K27me3 can contribute to differential gene expression under natural conditions, but it is not required, and additional regulatory mechanisms are likely important to increase DNA accessibility and enact transcription. An interesting finding in our research was the quantitative association we observed between H3K27me3 levels and transcriptional output between the growth conditions. This is interesting because it is not clear what mechanism explains a quantitative rather than qualitative change in H3K27me3 levels. That is, a nucleosome can have none, one, or two H3K27 tails with a tri-methyl modification, and quantitative differences seen in our samples reflect either heterogeneity in H3K27me3 status for cells in the population (e.g., 50% of cells are H3K27me3 and 50% are H3K27 non-modified) or it represents bivalent chromatin domains (e.g., 100% of cells have 50% H3K27me3). Bivalent domains are generally defined as DNA regions interacting with nucleosomes that contain chemical modifications representative of both active and repressed chromatin, with the prevailing example being the presence of both H3K4me3 and H3K27me3 (Blanco

et al., 2020). Bivalent domains have been identified and described in animal systems, and are thought to exist in a poised transcriptional state, with low steady state transcription capable of rapid transcriptional activation during cellular differentiation (Bernstein *et al.*, 2006; Voigt *et al.*, 2013). To our knowledge, bivalent domains have not been formally described in fungi. Whether our data are the result of a heterogenous population of cells or represent bivalent domains remains to be resolved. Nevertheless, our results show that many genes differentially expressed between growth conditions are associated with quantitative differences in H3K27me3 levels, providing evidence that chemical histone modifications dynamics can play a transcriptional regulatory mechanism in *V. dahliae*. As our experiments were based on differences between growth conditions, these results may represent a general regulatory mechanism for environmentally response genes.

At present, our conclusions are limited to the tested conditions, and it is possible that analyzing H3K27me3 levels in different cell-types or growth stages (e.g. spores, microsclerotia, *in planta* infection) may yield a different picture of global H3K27me3 distribution. Additionally, our experiments focused on steady-state growth on different media as these provide a clear and reproducible transcriptional difference. It is possible that H3K27me3 dynamics occur rapidly in response to environmental changes, cues, or developmental stages that we did not capture.

It appears evident that H3K27me3 contributes to additional genome functions beyond strict transcriptional repression, at least in fungi (Ridenour *et al.*, 2020). This is supported by our data showing the a majority of H3K27me3 domains were stable between the tested growth conditions, indicating these H3K27me3 domains have additional functions. These stable H3K27me3 domains may represent another form of constitutive heterochromatin, but this fact seems unlikely given that in our data many stable H3K27me3 domains harbored differentially expressed genes between growth media. Additionally, results in *N. crassa* have shown that genetic perturbation leading to the genome-wide loss of H3K9me3 causes a redistribution of H3K27me3 to previously H3K9me3 marked sites, but interestingly, this re-distribution does not result in

transcriptional silencing (Basenko *et al.*, 2015). Rather, the redistribution of H3K27me3 in *N. crassa* lacking H3K9me3 appears to contribute to genomic instability (Basenko *et al.*, 2015; Lewis, 2017). In *Z. tritici*, the enrichment of H3K27me3 at dispensable chromosomes and empirical evidence that H3K27me3 somehow increases genomic instability, additionally supports the hypothesis that H3K27me3 contributes to additional genomic functions beyond transcriptional regulation (Schotanus *et al.*, 2015; Möller *et al.*, 2019). Additional research is needed to fully understand the mechanisms of H3K27me3 targeting, dynamics, and impact on genome stability in fungi. The results presented here show that H3K27me3 domains are largely similar between *in vitro* growth conditions, but that quantitative differences in H3K27me3 levels are associated with concomitant transcriptional differences between the tested conditions.

MATERIALS AND METHODS

Fungal growth conditions

Verticillium dahliae strain JR2 (CBS 143773; Faino et al., 2015) was cultured on potato dextrose agar (PDA) (Oxoid, Thermo Scientific) at 22°C in the dark. Liquid cultures were obtained by collecting conidiospores from PDA plates after approximately 2 weeks of growth followed by inoculation at a final concentration of 1×10^4 spores per mL into liquid growth media. Media used in this study are potato dextrose broth (PDB) (Difco, Becton Dickinson, Franklin Lakes, NJ, USA), half strength Murashige & Skoog plus vitamins (MS) (Duchefa-Biochemie, Haarlem, The Netherlands) medium supplemented with 3% sucrose, and Czapec-Dox medium (CZA) (Oxoid, Thermo Scientific, Waltham, MA, USA). Liquid cultures were grown for 6 days in the dark at 22°C at 140 RPM. Mycelium was collected by straining cultures through miracloth (22 μ m) (EMD Millipore, Darmstadt, Germany) and pressing to remove liquid after which the mycelium was flash frozen in liquid nitrogen and ground to powder with a mortar and pestle. If required, samples were stored at -20°C prior to nucleic acid extraction. All analyses were performed based on triplicate cultures that were processed individually.

RNA sequencing and analysis

RNA was isolated from ground mycelium using TRIzol (Thermo Fisher Science, Waltham, MA, USA) following manufacturer guidelines. Contaminating DNA was removed using the TURBO DNA-free kit (Ambion, Thermo Fisher Science, Waltham, MA, USA) and RNA integrity was assessed with gel-electrophoresis and quantified using a Nanodrop (Thermo Fisher Science, Waltham, MA, USA). Library were prepared and singleend 50 bp sequenced on the DNBseq platform at BGI (Hong Kong, China).

Sequencing reads were mapped to the reference annotation of *V. dahliae* strain JR2 (Faino et al., 2015) using Kallisto quant (Bray et al., 2016) to calculate per gene TPM values. Differential expression between cultivation in PDB and CZA, MS or during colonization of *Arabidopsis thaliana* was determined using DESeq2 (Love et al., 2014).

H3K27me3-enriched regions were determined using epic2 with a binsize of 2,500 bp (Stovner & Sætrom, 2019).

ChIP-sequencing and analysis

ChIP-seq was performed as described previously (Cook et al., 2020). Frozen ground mycelium was added to ChIP lysis buffer, dounced 40 times and subsequently sonicated for 5 rounds of 20 seconds with 40 second rest stages on ice. Supernatants were collected after centrifugation and treated with MNase (New England Biolabs, Ipswich, MA, United States) for 10 minutes in a 37°C waterbath. MNase activity was quenched by addition of EGTA and samples were subsequently pre-cleared by addition of 40 µl Protein A Magnetic Beads (New England Biolabs, Ipswich, MA, United States) and rotating at 4°C for 60 min. Beads were captured and supernatant was divided over new tubes containing antibodies against either H3K4me2, H3K9me3 or H3K27me3 (ActiveMotif; #39913, #39765 and #39155) and incubated overnight with continuous rotation at 4°C. Subsequently, the antibodies were captured, washed and nucleosomes were eluted from beads, after which DNA was treated with Proteinase-K and cleaned-up using chloroform. DNA was isolated by overnight precipitation in ethanol and DNA concentration was determined with the Qubit™ dsDNA HS Assay Kit (Thermo Fisher Science, Waltham, MA, USA). Sequencing libraries were generated using the TruSeq ChIP Library Preparation Kit (Illumina, San Diego, CA, United States) according to instructions, but without gel purification and with use of the Velocity DNA Polymerase (BioLine, Luckenwalde, Germany) for 25 cycles of amplification. Single-end 125bp sequencing was performed on the Illumina HiSeq2500 platform at KeyGene N.V. (Wageningen, the Netherlands).

Raw reads were trimmed using TrimGalore (Martin, 2011) and mapped to the *V. dahliae* strain JR2 reference genome (Faino et al., 2015) using BWA-mem with default settings (Li, 2013). Three regions of the genome were masked due to aberrant mapping, (chr1:1-45000, chr2:3466000-3475000, chr3:1-4200). ATAC-seq reads of *V. dahliae* cultured in PDB (Cook et al., 2020) were treated similarly as ChIP-seq reads, but the paired end read pairs were trimmed and mapped simultaneously, and only read-pairs

<100 bp were considered for further analyses, as these represent open DNA. Mapped reads were RPGC-normalized using deepTools bamCoverage (Ramírez *et al.*, 2014) with binsize 1,000 and smoothlength 3,000 for plotting over the genome, and binsize 10 and smoothlength 30 for further analysis. Average coverage over gene bodies per expression quintile was calculated using deepTools computeMatrix in scale-regions mode (Ramírez *et al.*, 2014). Expression quintiles were generated by sorting all genes based on their average TPM value from three replicates of *V. dahliae* cultured for 6 days in PDB. To assess the presence of the histone marks over promoters (500 bp upstream of TSS) and gene bodies (region between TSS and TES) of genes located in LS regions and the core genome, we used deepTools multiBigwigSummary (Ramírez *et al.*, 2014) to calculate the average coverage of the histone marks and chromatin accessibility for each gene.

Changes in H3K27me3 levels was determined by calculating the average coverage over a gene using featureCounts (Liao *et al.*, 2014) on sorted bam files, and comparing the coverage between duplicates of cultivation in PDB and CZA or MS with DESeq2 (Love *et al.*, 2014). Changes in H3K27me3 and differential expression per gene were analysed.

Generation of *Set7* deletion mutant

The *Set7* deletion mutant ($\Delta Set7$) was constructed as previously described (Santhanam, 2012). Briefly, genomic DNA regions flanking the 5' and 3' ends of the coding sequences were amplified with PCR using primers listed in Table S1 (primers 1-4) and cloned in to the pRF-HU2 vector (Frandsen *et al.*, 2008) using USER enzyme following the manufacturer's protocol (New England Biolabs, MA, USA). Sequence-verified vectors were transformed into *Agrobacterium tumefaciens* strain AGL1 and used for *V. dahliae* conidiospore transformation as described previously (Santhanam, 2012). *V. dahliae* transformants that appeared on hygromycin B were transferred to fresh PDA supplemented with hygromycin B after five days. Putative transformants were screened using PCR to verify deletion of the target gene sequence and integration of the selection marker at the designated locus using primers listed in Table S1 (primers 5-8). Analysis

and comparison of gene expression in the *Set7* deletion mutant to type *V. dahliae* was performed as described above.

ACKNOWLEDGEMENTS

This work was supported by a PhD grant of the Research Council Earth and Life Sciences (project 831.15.002) to HMK, and by Human Frontier Science Program Postdoctoral Fellowship (HFSP, LT000627/2014-L), by USDA's National Institute of Food and Agriculture (award no. 2018-67013-28492) through the Plant Biotic Interactions Program, and by the National Science Foundation (award no. 1936800) Division of Molecular and Cellular Biosciences to DEC. Work in the laboratories of M.F.S and B.P.H.J.T. is supported by the Research Council Earth and Life Sciences (ALW) of the Netherlands Organization of Scientific Research (NWO). B.P.H.J.T acknowledges funding by the Alexander von Humboldt Foundation in the framework of an Alexander von Humboldt Professorship endowed by the German Federal Ministry of Education and Research is furthermore supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany's Excellence Strategy – EXC 2048/1 – Project ID: 390686111. The authors declare no conflict of interest.

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FIGURE LEGENDS

Figure 1. Genes that are higher expressed in CZA, MS and *in planta* than in PDB are overrepresented in adaptive genomic regions and in H3K27me3 domains.

Fractions of total genes and genes that are higher expressed than in PDB that locate in (A) adaptive genomic regions and (B) H3K27me3-enriched regions. Increased expression is determined between six days of cultivation *in vitro* of *V. dahliae* in potato dextrose broth (PDB) and 6 days of cultivation in Czapec-Dox (CZA) and half-strength Murashige Skoog medium (MS), and infection of *Arabidopsis thaliana* (21 dpi) (*in planta*). Fractions of higher expressed genes that reside in adaptive genomic regions or H3K27me3-enriched regions are reported for log2-fold changes >1, 2, 3, 4, 5, 6 and 7, respectively. H3K27me3-enrichment was determined for *V. dahliae* cultivation during six days in PDB. Black numbers above the bars indicate numbers of genes per gene set. White numbers at bottom of the bars indicate the numbers of genes that locate in adaptive genomic or H3K27me3-domain per gene set. Significance of fraction differences was calculated for each fraction of higher expressed genes in adaptive genomic regions when compared with the fraction of total genes in adaptive genomic regions, by the two-sided two-proportions Z-test (*: $p \leq 0.05$).

Figure 2. Lowly and non-expressed genes associate with H3K27me3.

A) Whole genome distribution of the euchromatin-associated histone modification H3K4me2, the constitutive heterochromatin-associated histone modification H3K9me3, the facultative heterochromatin-associated histone modification H3K27me3 and chromatin accessibility as determined by ATAC-seq, based on chromosome 5 as an example. Genes are indicated in light blue, transposons are indicated in red, centromere is indicated in dark blue and adaptive genomic regions are indicated in yellow. B,C) Relative coverage of chromatin accessibility and the histone marks H3K4me2, H3K9me3, H3K27me3 over gene bodies (between transcription start site (TSS) and transcription end site (TES)) ± 800 bp of flanking sequence, grouped into quintiles based on gene expression levels

upon cultivation for six days potato dextrose broth (PDB), for (B) all genes, and for (C) all H3K27me3-enriched genes.

Figure 3. Genetic perturbation of H3K27me3 results in induction of genes that are transcriptionally regulated in different growth conditions. A) Fractions of induced (log2 fold change >1, p<0.05) and repressed (log2 fold change <-1, p<0.05) genes and genes that are not differentially expressed between wild-type and Δ Set7 that (B) locate in H3K27me3 domains and (C-E) are higher expressed in CZA, MS and *in planta* than in PDB.

Figure 4. Differential H3K27 methylation partially explains differential expression. A) The number of genes located in H3K27me3 domains for *V. dahliae* cultivated for 6 days in Czapec-Dox (CZA), half-strength Murashige Skoog medium (MS) and Potato dextrose broth (PDB). B) Transcriptional values from RNA-seq, represented as TPM values in each growth medium. The genes were grouped between those consistently localized in H3K27me3 domains (1631 genes) and genes not present in H3K27me3 domains. C,D) Transcription TPM values in each growth medium for genes in H3K27me3 domains found in C) two or D) one of three tested *in vitro* growth media. Median values of violin plots are indicated with diamonds. Significant difference of TPM values between growth media are determined with the One-Sample Wilcoxon Signed Rank Test (*: p <= 0.05).

Figure 5. Differentially expressed genes associate with local changes in H3K27me3 coverage. Pairwise comparisons of differentially expressed genes for *V. dahliae* cultivated for 6 days in A,B) half-strength Murashige Skoog medium (MS) and Potato dextrose broth (PDB), C,D) Czapec-Dox (CZA) and PDB, and E,F) MS and CZA. A,C,E) H3K27me3 coverage over differentially expressed genes in each corresponding growth medium. Median values of violin plots are indicated with diamonds. B,D,F) per gene comparison of log2-fold change in transcription and genic H3K27me3 coverage.

Black horizontal lines indicate TPM log₂-fold change of -2 and 2, black vertical lines indicate H3K27me3 log₂-fold change of -1 and 1. Significant difference of TPM values between growth media are determined with the One-Sample Wilcoxon Signed Rank Test and indicated by letters or by asterisks (*: p≤0.05).

Figure 6. *In planta*-induced genes are H3K27me3-associated under transcriptionally repressive conditions. Average coverage of chromatin features over genes differentially expressed between *in planta* and A) 6 days of cultivation in Potato dextrose broth (PDB), B) half strength Murashige Skoog medium (MS), and C) Czapek-Dox medium (CZA). D) overlap of genes that are higher expressed *in planta* than in PDB, MS or CZA with genes located in an H3K27me3 domain in the corresponding growth medium. Significant differences of H3K27me3 coverage are determined with the One-Sample Wilcoxon Signed Rank Test (*: p ≤ 0.05).

ADDITIONAL FILES

Fig. S1. Distribution of chromatin features over all chromosomes. Whole genome distribution of the euchromatin-associated histone modification H3K4me2 (green line), the constitutive heterochromatin-associated histone modification H3K9me3 (red line), the facultative heterochromatin-associated histone modification H3K27me3 (yellow line) and chromatin accessibility (blue line) as determined by ATAC-seq. Genes are indicated in light blue, transposons are indicated in red, centromere is indicated in dark blue and adaptive genomic regions are indicated in yellow.

Fig. S2. Gene expression correlates with histone modification presence and chromatin accessibility. RPGC normalized coverage of the histone marks H3K4me2, H3K9me3, H3K27me3 and chromatin accessibility over gene bodies (between transcription start site (TSS) and transcription end site (TES)) ± 800 bp of flanking sequence. Each row represents a single gene, and are sorted based on their TPM value upon cultivation for six days potato dextrose broth (PDB), with the top gene being most highly expressed. Genes are grouped in expression quintiles as in Figure 3A.

Fig. S3. The *V. dahliae* $\Delta Set7$ deletion mutant loses H3K27me3. Histone isolations of wild-type, $\Delta Dim2$, $\Delta Dim5$, ΔHPI and $\Delta Set7$ were tested for presence of the H3K27me3 histone modification by Western blot. Antibody against H3 was used as loading control.

Fig. S4. Genes associated with H3K27me3 in wild type *V. dahliae* are stronger transcriptionally induced in the $\Delta Set7$ mutant than genes that are not associated with H3K27me3. Log2-fold change of expression between wild type and $\Delta Set7$ mutant for genes associated with H3K27me3 in wild type (green) and those not associated with H3K27me3 in wild type (red). Significant difference of log2-fold change between gene sets are determined with the One-Sample Wilcoxon Signed Rank Test (*: $p \leq 0.05$).

Fig. S5. Distribution of H3K27me3 for *V. dahliae* cultivated in *in vitro* growth media. Replicates of *V. dahliae* cultivated for 6 days in Potato dextrose broth (PDB), half

strength Murashige Skoog medium (MS) and Czapec-Dox medium (CZA). A) Distribution of H3K27me3 over the *V. dahliae* genome, predicted H3K27me3 domains are indicated as blocks below each H3K27me3 track. Adaptive genomic regions are highlighted in yellow. B) Distribution of H3K27me3 over chromosome 5 of *V. dahliae*. Genomic regions within H3K27me3 domains that are visibly different between growth conditions are highlighted in grey.

Table S1. Primers used to delete and analyse the *Set7* coding sequence in *V. dahliae*.