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Research Article

The Histone Deacetylase Inhibitor Trichostatin-A Modifies the Expression of Trichothecene Mycotoxin Regulatory Gene *Tri5* in *Fusarium graminearum*

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Background: *Fusarium graminearum* is the causal agent of Fusarium Head Blight (FHB) on wheat and produces deoxynivalenol (DON), known to cause extreme human and animal toxicosis. This species' genome contains genes involved in plant-pathogen interactions and regulated by chromatin modifications. Moreover, histone deacetylase inhibitors (HDACIs), including trichostatin A (TSA), have been employed to study gene transcription regulation because they can convert the structure of chromatin.

Objectives: The current study was designed to evaluate the effects of TSA on histone deacetylase (*HDAC*) and, trichodiene synthase (*Tri5*) gene expression in toxigenic and non-toxigenic *F. graminearum* isolates.

Materials and Methods: The mycelia were grown on potato dextrose broth (PDB) culture media supplemented with two concentrations of TSA and dimethyl sulfoxide (DMSO) (3 and 10 µg. mL^{-1}) for 48 h, 72 h, and 96 h. Then, the mRNA levels were estimated via real-time quantitative reverse transcription-polymerase chain reaction (real-time qRT-PCR).

Results: We found that the levels of *HADC* and *Tri5* varied over time and dosage in response to the use of TSA. The toxigenic isolate showed an increase in the *Tri5* expression when treated with TSA, with the highest levels monitored when the concentration of the substance was 3 µg. mL^{-1} at 48 h. The non-toxigenic isolate also showed high levels of *HDAC* and *Tri5* expression in the presence of TSA, but a sharp decrease in the *Tri5* transcription was observed at 72 h when grown on culture media containing 10 µg. mL^{-1} of TSA.

Conclusion: Overall, our results suggest a mode of DON biosynthesis regulation in *F. graminearum* by chromatin modifications that may help us offer new strategies for tackling fungal infections.

Keywords: Chromatin, Deacetylation, Deoxynivalenol, Epigenetics, Fungi

1. Background

Fusarium graminearum Schwabe [teleomorph: *Gibrella zea* (Schweinitz) Petch] is known as the primary causal agent of Fusarium Head Blight (FHB) throughout the world, leading to a substantial reduction in the yield of cereal crops under favorable environmental conditions (1-4). It produces highly hazardous mycotoxins, including deoxynivalenol (DON), an essential virulence

factor to facilitate the infection development of the pathogen in host plants (5, 6). The fungus produces DON through a genetic locus consisting of two genes (*Tri1-Tri16*), a core cluster of 12 genes, and a single-gene locus *Tri101*. Seven of these genes (*Tri8, Tri7, Tri3, Tri4, Tri5*, *Tri11*, and *Tri13*) encode enzymes that catalyze ten reactions in the biosynthesis of trichothecene. The first step in the DON biosynthesis process is carried out by trichodiene synthase (*Tri5*), which converts farnesyl diphosphate into trichodiene through cyclization (7). The cytochrome P450 oxygenase (*Tri13*) gene encodes trichothecene-15-O-acetyltransferase (15-ADON); however, this gene can be a pseudogene due to the accumulation of mutations over time, leading to the loss of its ability to produce a functional protein. *F. graminearum* isolates with a functional *Tri13* gene can produce both DON and Nivalenol (NIV), whereas isolates that carry the *Tri13* pseudogene produce DON but not NIV. As a result, scientists use this pseudogene to determine whether a particular isolate produces DON (7).

In fungi, genes involved in secondary metabolism are co-localized into clusters to facilitate horizontal gene transfer and transcriptional regulation (8-10). The accessibility of secondary metabolite gene clusters to transcriptional elements is determined by chromatin structure. Therefore, histone modifications influencing chromatin dynamics may help regulate these clusters in response to external stimuli (11, 12). Reversible histone acetylation is a renowned epigenetic modification that changes gene expression by chromatin remodeling. This modification is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs) (8, 13, 14). Generally, HATs act as co-activators of gene transcription not only by weakening the interaction between DNA and histones but also by making a docking surface for other regulatory components (12), but HDACs cause gene silencing as a result of removing acetyl groups from acetylated lysine residues in the tail of core histones (15). On the other hand, some research has illustrated the necessity of HDACs in gene transcription. For example, transcription of the polyketide synthase (*MpksCT*) gene, involved in Citrinin biosynthesis in *Monascus ruber,* is down-regulated in response to Dihydrocoumarin (an inhibitor of the Sirtuin family of NAD^+ -dependent deacetylase) (16).

The central role of fungal HDACs in different cellular processes, such as survival, pathogenicity, normal growth, and vital metabolite production, has made these enzymes promising targets for HDACs inhibitors (HDACIs). HDACIs are a novel group of drugs to address diseases because they can change transcription and induce apoptosis, cell cycle arrest, and differentiation (11, 14, 17-20). To cite an example: The gene expression patterns of *Aspergillus clavatus, Fusarium verticillioides,* and *Aspergillus nidulans* significantly adjusted when trichostatin A (TSA) was added to their culture media (8, 11, 21). TSA is an HDACI initially isolated from *Streptomyces hygroscopicus* that blocks the catalytic sites of class I and Π HDACs by imitating lysine and chelating the zinc atom necessary for the deacetylation reaction (22-25).

The field of epigenetics deals with adjustments in gene expression that occur without altering the DNA sequence (26). These changes happen in response to the environment and can lead to the emergence of specific phenotypes. Epigenetic modifications may have the potential to improve disease management, reproductive success, productivity, and environmental adaptation in eukaryotes (27). Over the past few years, several studies have been conducted on HDACs in *F. graminearum* through mutation and deletion approaches (1, 5, 28, 29); however, no HDACIs have been tested to treat this species so far.

2. Objectives

In this project, our aim was to investigate the impact of TSA on the expression of *HDAC* and *Tri5* genes in *F. graminearum*. Additionally, we wanted to evaluate the responses of both DON-producing and non-DON-producing isolates.

3. Materials and Methods

3.1. Fungal Isolates and Chemical Compound

Fifteen isolates of *F. graminearum* were chosen from the fungal collection at the Science and Research Branch, Islamic Azad University, Tehran, Iran **(Table 1).** Single-spore isolates grown on Potato Dextrose Agar (PDA) were incubated at 25 °C for seven days. Next, molecular identification of these isolates was confirmed with species-specific primers (unpublished results).

Table 1. The codes and the origins of the isolates used in this study

Code	Collection code	Region	Province
1	2507	Qara Kheyl	Mazandaran
2	2509	Qara Kheyl	Mazandaran
3	2510	Qara Kheyl	Mazandaran
4	2511	Iraghi Mahaleh	Golestan
5	2512	Qara Kheyl	Mazandaran
6	2513	Iraghi Mahaleh	Golestan
7	2515	Iraghi Mahaleh	Golestan
8	2517	Iraghi Mahaleh	Golestan
9	2518	Iraghi Mahaleh	Golestan
10	2519	Iraghi Mahaleh	Golestan
11	2520	Iraghi Mahaleh	Golestan
12	2521	Sorkhan Kelaate	Golestan
13	2522	Now Deh-e Malek	Golestan
14	2523	Kamalabad	Golestan
15	2524	Kalaleh	Golestan

TSA was purchased from Sigma-Aldrich (St. Louise, USA) and dissolved in 3.333 mL of Dimethyl sulfoxide (DMSO). The stock solution was stored at -20 °C.

3.2. Primers

The characteristics of the primers used in this study are presented in **Table 2**. National Center for Biotechnology Information (NCBI), MEGA X, and Primer3 (v. 0. 4. 0) software programs (30, 31) were used to design those that targeted *HDAC*.

3.3. HDAC, Tri5, and Tri13 Genes Detection

In the first step, seven-day-old mycelia cultured on PDA at 25 °C were harvested and crushed to a fine powder in liquid nitrogen. Then, total genomic DNA (gDNA) was extracted using the CTAB method (32) and stored at -20 °C. Following this, Polymerase chain reaction (PCR) assays were carried out by specific primers using a T100 thermal cycler (Bio-Rad, USA). The thermal program for *HDAC* consisted of 30 s at 94 °C to initiate the reaction, followed by 30 cycles of 95 °C for 30 s; 55 °C for 30 s; 72 °C for 3 min, and a final extension step (72 °C for 60 s). The PCR program for *Tri5* included a 75 s step at 94 °C to initiate the reaction, 30 cycles (94 °C for 15 s; 60 °C for 30 s; 72 °C for 45 s), and a final extension step (72 °C for 255 s) (33).

We screened the *Tri13* pseudogene in all isolates with specific primers (Tri13F/Tri13DONR) (2). The PCRs were performed according to the following program: 5 min at 94 °C, followed by 35 cycles of 94 °C for 60 s, 58 °C for 45 s, 72 °C for 60 s, and a final extension of 72 °C for 5 min (2).

Each PCR experiment was accomplished in three independent replications, and its products were separated by electrophoresis through one percent agarose gels.

3.4. Treatment

In the first stage of the process, mycelia were grown in 200 mL of Potato Dextrose Broth (PDB) for seven days. The growth was carried out at room temperature in the dark with orbital shaking (130 g). Next, the seven-day-old mycelia were transferred to 5 mL of PDB enriched with two different concentrations of TSA (3 and 10 µg. mL^{-1}). Negative control samples were prepared using DMSO at 3 and 10 µg. mL^{-1}, depending on the TSA concentration in the experimental samples. The selected concentrations of TSA were based on previous research (20) and our morphological study (unpublished data). The culture media were

Table 2. Specifications of the primers used in this project

Primer	Accession Number	Sequence (5'➔3')	Size (bp)	Tm (°C)	Reference
Tri5F	NC_026474	AGCGACTACAGGCTTCCCTC	545	62	Nicholson *et al*. (2004)
Tri5R		AAACCATCCAGTTCTCCATCTG			
HDACF	NC_026474	GCGACGACATTCGTGTTGAG	346	62.83	This study
HDACR		TCTGGCGTGACTTTTTGCAG		62.46	
Tri13F	AF336366	CATCATGAGACTTGTKCRAGTTTGGG	282	66	Rezaeian Doloei *et al.* (2015)
Tri13DONR		GCTAGATCGATTGTTGCATTGAG		61	
Real-time RT-PCR					
UBHF	NC_026474	GTTCTCGAGGCCAGCAAAAAGTCA	168	62.4	Kim *et al.* (2011)
UBHR		CGAATCGCCGTTAGGGGTGTCTG		65.2	
Tri5F	NC_026474	TTTCCCACCGAGTATTTTCT	170	61	Zheng *et al.* (2017)
Tri5R		TAGGGTCTACCTTGAGCATCT		64	
HDACF	NC_026474	TCGGTGGAGGTGGCTATACA	154	61.46	This study
HDACR		GTTTGAGGATCGGACGTTCA		61.18	

then kept in the dark with orbital shaking at 130 g for 48 h, 72 h, and 96 h. The experiment was repeated three times independently. The mycelia were filtered through sterile cellulose filters and pulverized into a fine powder for RNA isolation.

3.5. RNA Extraction

The RNA extraction was carried out using a BioFACT Total RNA Prep Kit (ver.2.0) (Daejeon, Korea), following the established protocol for plant tissue. RNA concentration and purity were evaluated on a Nanodrop (Thermo Scientific, USA). RNA unity was examined using a 0.8 percent agarose gel.

3.6. RT-PCR

After subjecting RNA samples to DNase I, as stated in the Thermo Fisher Scientific protocol, their concentrations and purities were evaluated on a Nanodrop. Next, a control PCR was done with 4 µL of total RNA and Ubiquitin carboxyl-terminal hydrolase (*UBH*) primers (34), with no reverse transcription to check for gDNA contamination. The thermal program comprised 30 s at 95 °C to initiate the reaction, followed by 35 cycles of 95 °C for 30 s; 54 °C for 30 s; 72 °C for 60 s, and a final extension step (72 °C for 3 min). PCR assays were repeated three times for each sample, and their products were separated on one percent agarose gels by electrophoresis.

Reverse transcription of RNA samples was conducted using a cDNA Synthesis Kit (Yekta Tajhiz Azma, Iran) and Oligo dT primers. Next, the primers for *Tri5* (35), *HDAC,* and *UBH* were used to check the quality of the retrotranscription through PCRs. Cycles were programmed for the *HDAC* and *Tri5* as follows: one initial denaturing cycle at 95 °C for 30 s, 35 cycles (95 °C for 30 s; 54 °C for 30 s (57 °C for *Tri5*); 72 °C for 60 s), and a final extension step (72 °C for 3 min). The PCR tests were repeated three times, and the products were separated by electrophoresis through one percent agarose gels.

3.7. Quantitative, Real-time RT-PCR

Real-time qRT-PCR assays were performed using a

Real-time thermal cycler (Corbett Rotor-gene 6000) (Qiagen, USA). PCRs were accomplished in a 10 µL solution containing 1 µL (120 ng) of the reverse transcription product, 0.2 µL of each primer (for *HDAC,* it was 0.3 µL), and 5 µL RealQ Plus 2x Master Mix Green (Ampliqon, Denmark). The thermal cycle protocol was 95 °C for 10 min, followed by 35 cycles (40 cycles for *Tri5*) of 95 °C for 30 s, 58 °C (52 °C for *HDAC*) for 30 s, and 72 °C for 15 s (10 s for *HDAC*) followed by a melting curve analysis from 72-95 °C. Each RT-qPCR run was conducted in two replications. The *UBH* transcript levels were used as a reference to normalize the output. Relative expression of the genes was calculated by the Livak method (36).

3.8. Statistical Analysis

Real-time PCR data was analyzed using LinReg and REST KIAGENE 2009 software (37, 38). CT and reaction efficiency were determined using LinReg PCR software, while REST software was used to compare the expression changes of *Tri5* and *HDAC* genes between the control and treatment groups. The significance of the results was determined using the SPSS program and *t*-test. $P < 0.01$ and $0.01 < P < 0.05$ were considered significant. Additionally, the GraphPad PRISM program was used to create graphs (39).

4. Results

4.1. HDAC, Tri5, Tri13 Detection

PCRs were performed using specific primers to detect *HDAC*, *Tri5,* and *Tri13* genes in *F. graminearum* isolates. The size of PCR products, amplified by three pairs of primers, was 347 bp, 545 bp, and 282 bp sequentially **(Fig. 1A, 1B and Fig. 2).**

4.2. Transcripts Detection

Minus-reverse transcriptase PCR (by omitting the reverse transcriptase addition step) was performed using *UBH* primers to assess the amount of DNA contamination present in RNA samples. The lake of expected PCR amplicons (168 bp) confirmed that there is no gDNA in RNA samples **(Fig. 3A)**. Then, Reverse transcription PCRs (RT-PCRs) were done to amplify respective transcripts from complementary DNA (cDNA) samples. *UBH, HDAC,* and *Tri5* primers provided the expected amplification products belonging to 168 bp, 154 bp, and 170 bp sizes in the order already mentioned **(Fig. 3B).** The results showed that the primers used to amplify gene fragments are specific.

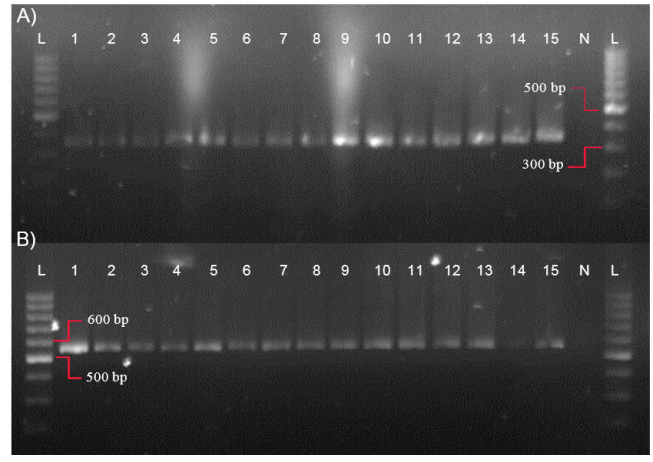

Figure 1. Electrophoretic pattern of PCR products for *F. graminearum* isolates. A) Using HDACF/HDACR primers. L: 100 bp molecular weight marker (Thermo Scientific, USA); N: Negative control (without DNA template); 1-15: Different isolates of *F. graminearum.* **B)** Using Tri5F/Tri5R primers. L: 100 bp molecular weight marker (Thermo Scientific, USA); N: Negative control (without DNA template); 1-15: Different isolates of *F. graminearum.*

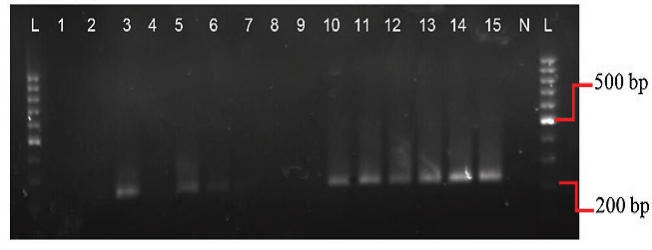

Figure 2. Electrophoretic pattern of PCR products for *F. graminearum* isolates using Tri13F/Tri13DONR primers. L: 100 bp molecular weight marker (Thermo Scientific, USA); N: Negative control (without DNA template); 1-15: Different isolates of *F. graminearum*

4.3. Trichostatin A Affected Gene Expression

4.3.1. HDAC

After analyzing the data, it was demonstrated that the expression of the *HDAC* gene was affected by TSA at two different concentrations (3 and 10 µg. mL^{-1}) in *F. graminearum*. In the toxigenic isolate, a concentration of 3 µg. mL^{-1} of TSA suppressed the gene expression (0.294 times) at 48 h but stimulated it (3.583 times) at 72 h compared to the control. Nevertheless, at 96 h, there were no significant statistical changes in the *HDAC* mRNA level (0.789 times) in response to TSA (3 µg. mL^{-1}). On the other hand, The *HDAC* expression escalated by 2.014-fold at 48 h in the presence of 10 µg. mL^{-1} of TSA, but the transcription adjusted no more than 0.783 times at 72 h and 0.832 times at 96 h compared to the control (**Fig. 4**).

In the non-toxigenic isolate, the expression of *HDAC* was found to be up-regulated by 4.955 and 4.901 times at 48 h and 96 h, respectively, when treated with 3 µg. mL^{-1} of TSA as compared to the control. However, at 72 h, the effect of TSA on the gene expression was negligible, with only a 1.604-fold increase. When treated with 10 µg. mL^{-1} of TSA, the transcription of *HDAC* rose by 2.826 times at 48 h, but no significant statistical changes were observed at 72 h and 96 h (**Fig. 4**).

4.3.2. Tri5

In the presence of 3 µg. mL^{-1} of TSA in the culture medium, the toxigenic isolate showed a noticeable increase in *Tri5* gene expression at 48 h and 96 h. The growth observed was respectively 381.357 and 175.460 times higher than the control. Under the same conditions, at 72 h, the expression was down-regulated to 0.493 times as compared to the control. Moreover, the statistical analysis indicated that the *Tri5* mRNA level at 48 h seriously declined to 0.036 times when the isolate was treated with a concentration of 10 µg. mL^{-1} of TSA. Although *Tri5* transcription encountered a vast boost (141.534 times) in response to 10 µg. mL^{-1} of TSA at 96 h, its slight pickup at 72 h was not statistically significant compared to the control (**Fig. 5**).

In the non-toxigenic isolate, we did not see any apparent modifications in the expression of *Tri5* at 48 h after exposure to 3 µg. mL^{-1} of TSA. In addition, we noticed an up-regulation in transcription by 3.317 and 3.375-fold at 72 h and 96 h, respectively. On the exposure to 10 µg. mL^{-1} of the HDAC inhibitor, we found that the gene expression decreased to 0.087 times at 72 h. However, these changes were

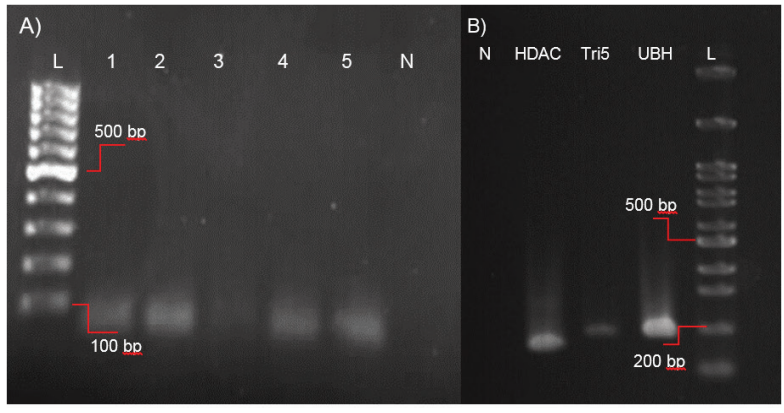

Figure 3. Reverse transcription PCRs (RT-PCRs) of respective genes. A) Minus reverse transcriptase PCR (without RT enzyme) products of *UBH* gene for *F. graminearum* isolates treated with TSA. L: 100 bp molecular weight marker (Thermo Scientific, USA); N: Negative control (without RNA template). 1-5: Different RNA samples of *F. graminearum*. **B)** RT-PCR products of *Tri5*, *HDAC*, and *UBH* genes for *F. graminearum* isolates treated with TSA. L: 100 bp molecular weight marker (Sinaclon, Iran); N: Negative control (without cDNA template).

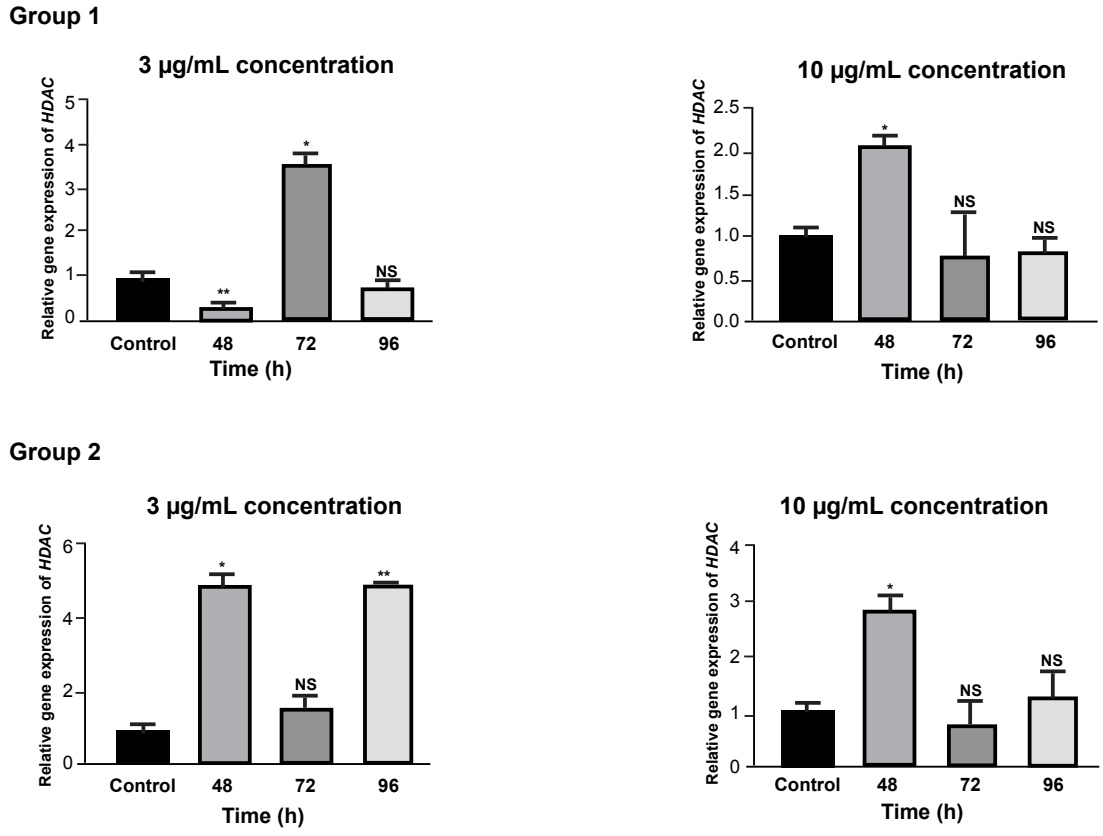

Figure 4. The effects of two different concentrations of TSA on the expression of *HDAC*, in toxigenic (group 1) and non-toxigenic (group 2) isolates of *F. graminearum* at 48 h, 72 h, and 96 h. The significant difference was determined by $p < 0.01^{**}$, $0.01 < p < 0.05^{*}$ vs. control. A horizontal line appears on the statistical comparison bar, showing no significant difference (NS)

statistically insignificant compared to the control at 48 h and 96 h (**Fig. 5**).

5. Discussion

Fungicides are widely used in agriculture to protect crops from fungal plant pathogens and ensure healthy, high-quality produce for the growing population (40). However, excessive and prolonged use of these chemicals can lead to the development of pathogenic resistance (40, 41). Fungal-resistant isolates are less sensitive to the commonly used fungicides becoming dominant under selection pressure (41). Hence, it is essential to develop natural and efficient chemicals to address the challenge of food safety (40).

Eukaryotic HDACs play a critical role in physiological and pathological conditions and can be promising candidate targets for HDACIs. These compounds are discussed as potential antifungal drugs since they can alter gene expression patterns by influencing gene promoters or other secondary and downstream effectors (42). The involvement of HDACs in fungal gene regulation was first demonstrated in 2001 through a study targeting the histone deacetylation complex 1 (*HDC1*) gene in *Cochliobolus carbonum.* The study revealed that *HDC1* mutants were unable to infect maize due to reduced penetration efficiency (13). Following this discovery, the significance of HDACs in fungal species was further investigated. For example, the deletion of histone deacetylase (*hdaA)* in *Aspergillus nidulans* raised the production of Sterigmatocystin and Penicillin. In addition, isopenicillin N synthase (*ipnA*) and isopenicillin N acyltransferase (*penDE*) genes up-regulated at 24 h, in contrast to versicolorin reductase (*stcU*) and aflatoxin regulatory (*aflR*) genes, which increased at 36 h (43). Prompted by evidence, we evaluated the

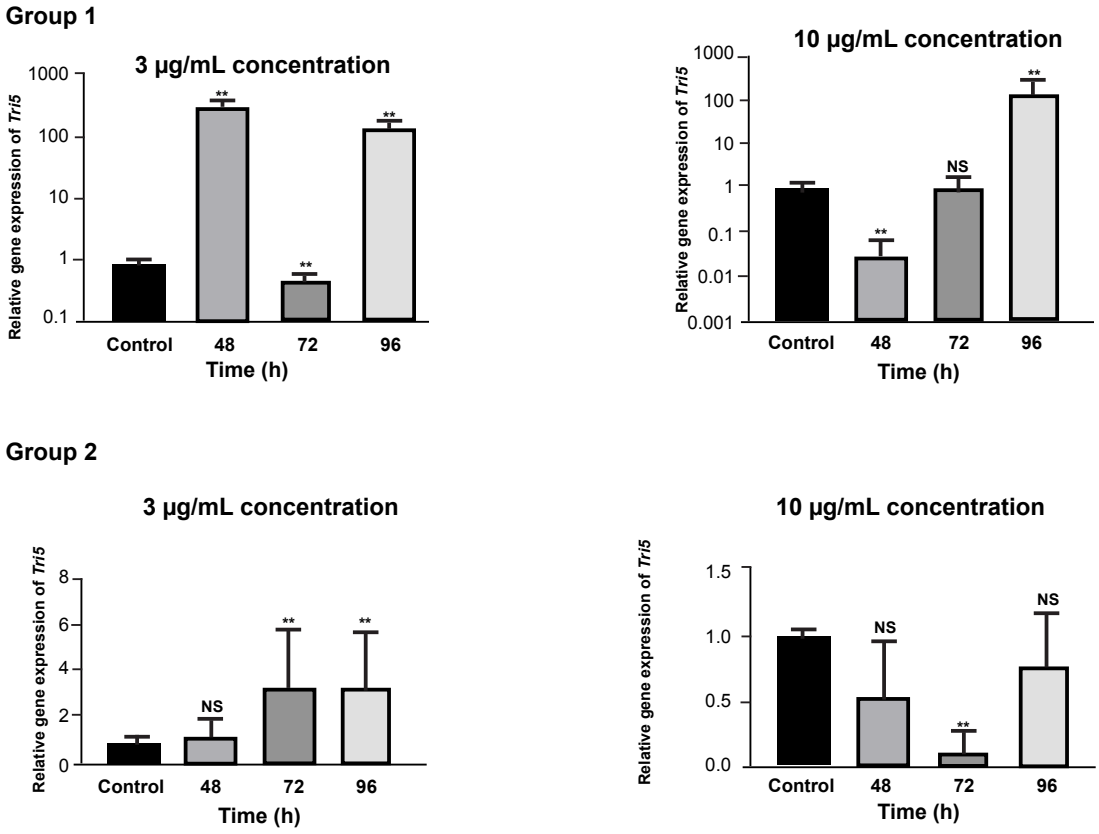

Figure 5. The effects of two different concentrations of TSA on the expression of *Tri5*, in toxigenic (group 1) and non-toxigenic (group 2) isolates of *F. graminearum* at 48 h, 72h, and 96 h. The significant difference was determined by $p < 0.01^{**}$, $0.01 < p < 0.05^{*}$ vs. control. A horizontal line appears on the statistical comparison bar, showing no significant difference (NS)

possible effects of TSA on the expression of *HDAC* and the regulatory gene *Tri5* in both DON-producing and non-DON-producing *F. graminearum* isolates. Our results indicated that TSA could change *HDAC* and *Tri5* gene expression patterns, but its performance depended entirely on the time and dose. For this project, we selected two *F. graminearum* isolates for qRT-PCR testing, one that produced DON and another that did not. The selection was based on the presence of the *Tri13* pseudogene, which is involved in the DON biosynthetic pathway. This molecular selection has been previously checked by HPLC analysis, as reported by Rezaee in 2005 (44) for these isolates.

Application of TSA (3 µg. mL^{-1}) considerably reduced *HDAC* expression at 48 h in the toxigenic *F. graminearum* isolate. However, its inhibitory effects were not as strong at 72 h and 96 h. In other words, TSA had profound impacts in the early stages of fungal development, but its efficiency diminished after 48 h. This may show the limited stability of TSA in fungal culture media (21). We also observed that the expression of the *Tri5* gene was highest at 48 h when exposed to 3 µg. mL^{-1} of TSA. On the other hand, *HDAC* expression was remarkably up at 48 h when exposed to a concentration of 10 µg. mL^{-1} of TSA, but the *Tri5* mRNA level was down at this concentration and time point. For that reason, in the DON-producing isolate, *HDAC* might contribute to the transcriptional repression of *Tri5,* and hyperacetylation significantly led to its catalytic activity reduction. These findings were consistent with those of previous studies. For instance, TSA enhanced the expression of fumonisin polyketide synthase (*FUM1)* and Zn (II)2 Cys6 DNA-binding protein (*FUM21*) genes in *F. verticillioides* (8). In *A. clavatus,* expression of polyketide synthase-non-ribosomal peptide synthetase (*ccs*A) and PKS/NRPS

hybrid synthetase (*psoA)* genes mounted in response to TSA and Valproic acid (VPA) at 48 h but fell at 72 h (21). Similarly, a study on *A. nidulans* demonstrated that the expression of orsellinic acid synthase (*orsA*), tyrosinase-like protein orsC (*orsC),* and dehydrogenase orsE (*orsE*) genes climbed on the culture media containing TSA after three hours (11).

In the non-toxigenic isolate of *F. graminearum*, the expression of *HDAC* was high when treated with TSA at concentrations of 3 and 10 µg. mL^{-1}. The use of TSA (3 µg. mL^{-1}) also caused an increase in the transcription of the *Tri5* gene. As a result, *HDAC* may function as a transcriptional activator in *Tri5* transcription by deacetylation of histones, binding directly to the *Tri5* gene sequence, or becoming a member of a functional complex (39). These findings are supported by similar observations that HDACIs diminished the expression of *MpksCT* and *ipnA* genes in *M. ruber* and *A. nidulanse,* respectively (16, 19). Besides, the absence of histone deacetylase *F. graminearum* 1 (*HDF1*) and heterochromatin protein 1 (*Hep1*) genes lowered the production of DON in *F. graminearum* (1, 5). At 72 h, the application of 10 µg. mL^{-1} of TSA resulted in significant inhibitory effects on *Tri5*. These outcomes suggest that *HDAC* in *F. graminearum* can activate or arrest transcription. These findings highlight the importance of regulating the acetylation/deacetylation cycle for proper gene expression in *F. graminearum* (45).

Morphological evidence confirmed the results obtained from our molecular data. We found that TSA affected the radial growth and asexual reproduction of both toxigenic and non-toxigenic isolates on PDA. The effect of TSA was dependent on its concentration and the duration of exposure (unpublished results). Similar adjustments in phenotype were observed in other species where HDACs were deleted. In *Aspergillus terreus,* NAD^+-dependent histone deacetylase (*HstD*) deletion induced less conidiation, vegetative growth, and mycelia branching on culture media than the control (46). Correspondingly, in *Macrophomina phasseolina,* TSA generated a fall-off in colony growth and microsclerotia size on solid media, and the mycelium showed a less pigmented appearance than the control (25).

6. Conclusion

In conclusion, the present study revealed that post-translational histone modifications are employed by *F. graminearum* to regulate the expression of genes involved in the trichothecenes biosynthesis pathway. This investigation may provide useful data for us to expand our molecular knowledge to identify novel fungus-specific drugs.

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CRediT authorship contribution statement

All authors contributed to the study's conception and design. Shiva Amin: Methodology, Investigation, Validation, Formal analysis, Resources, Data curation, Writing-Original draft, and Editing; Saeed Rezaee: Supervisor, Methodology, and Reviewing; Amir Mousavi: Supervisor, Methodology, Editing, and Reviewing; Hamidreza Zamanizadeh: Advisor and Reviewing. All authors have approved the manuscript.

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Data Availability

The data used or analyzed during this study are included in this article and available from the corresponding authors upon reasonable request.

Declariations

Competing Interest: The authors declare that there are no competing interests associated with this manuscript to report.

Ethic statement

This article does not contain any studies with human participants or animals performed by any of the authors.

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