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# An Acidic pH is a determinant factor for TRI genes expression and trichothecenes B biosynthesis in *Fusarium graminearum*

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**An Acidic pH is a determinant factor for TRI genes expression and trichothecenes B biosynthesis in *Fusarium graminearum***

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3 **An acidic pH is a determinant factor for *TRI* genes expression and**  
4 **trichothecenes B biosynthesis in *Fusarium graminearum***  
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## Abstract

Reducing production of type B trichothecenes by *Fusarium graminearum* on cereals is necessary to avoid contamination leading to yield reduction and harmful impacts on human and animal health. Understanding how trichothecenes biosynthesis is induced is essential. The effect of ambient pH on fungal growth, toxin biosynthesis and *TRI* genes expression was studied during *in vitro* liquid culture of *F. graminearum* on minimal medium. Fungal development stopped at day 3 after a sharp pH drop in the medium. At the same time, induction of *TRI* genes expression was observed and toxin started to accumulate one day later. Acidification seems a determinant factor for induction, as neither the toxin nor the *TRI* genes were detected when the pH was maintained neutral. Shifting from neutral to acidic pH by mycelium transfer induced *TRI* genes expression and toxin accumulation. The regulation of toxin production by the ambient pH seemed to be specific to some *TRI* genes since *TRI5*, located in the core *FgTRI5* cluster, showed an immediate induction while *TRII01*, located elsewhere in the genome, showed a more progressive response. The regulation of trichothecene biosynthesis by the ambient pH seems to be a general mechanism, independent from strains or chemotype as all tested strains, including *F. graminearum* and *F. culmorum* species, showed a regulation of toxin production in response to the ambient pH. We conclude that *in vitro*, external acidification is required for induction of *TRI* genes expression.

**Keywords:** *Fusarium*, trichothecenes, *TRI* genes, pH.

## Introduction

*Fusarium graminearum* (Teleomorph: *Gibberella zeae* Shwabe) is one of the causal agents of *Fusarium* diseases which infect many crop plants (Goswami and Kistler, 2004). *Fusarium* Head Blight and *Fusarium* Ear Rot, the most common *Fusarium* diseases on wheat and maize respectively cause yield losses and reduction in grain quality due to the accumulation of trichothecenes mycotoxins (Bhatnagar et al., 2002, Bennett and Klich, 2003). *F. graminearum* produces type B trichothecenes (TCT B) including deoxynivalenol (DON), nivalenol (NIV), and their acetylated derivatives (3ADON, 15ADON, 4ANIV) (Xu and Berrie, 2005). The trichothecene biosynthesis pathway was established and nearly all *TRI* genes that are implicated have been described in *Fusarium sporotrichioides* and *F. graminearum* (Desjardins et al., 1993, Lee et al., 2002, Kimura et al., 2003, Kimura et al., 2007). Most of them localize in one genomic locus which is now referred as the “*FgTRI5* cluster” (Hohn et al., 1993, Brown et al., 2004). Among the clustered genes, *TRI5* encodes the trichodiene synthase responsible for the first step in the pathway (Hohn and Beremand, 1989), *TRI4* encodes a multifunctional oxygenase which catalyses all the steps leading from trichodiene to isotrichotriol (Hohn et al., 1995, Tokai et al., 2007), *TRI6* gene encodes a Zinc finger transcription factor, it was found to be a positive regulator of other *TRI* genes (Proctor et al., 1995), *TRI6* itself is regulated by another regulatory protein encoded by *TRI10* (Tag et al., 2001). Two other *TRI* genes have been found in a two-gene minicluster, the *TRI1* cluster (Brown et al., 2003, McCormick et al., 2004) and another gene *TRI101* encoding a 3-O-acetyltransferase which catalyses the acetylation of trichothecenes resides outside the *TRI* gene clusters. Although this gene encodes an enzyme essential in the progression of the biosynthetic steps it has been described to have a key role in self-protection (Kimura et al., 1998).

Since the publication of the regulatory role of *TRI6* and *TRI10*, quite little is known about the induction of trichothecene production. A recent report showed that *TRI6* and *TRI10* controlled many fungal genes expression *in planta* (Seong et al., 2009). Thus, significant progress has to be made towards understanding the essential steps in the regulation of *TRI* genes induction. Some reports described a profound effects on trichothecenes production *in vitro* of carbon and nitrogen sources (Miller and Greenhalgh, 1985, Jiao et al., 2008) or fungicides (Covarelli et al., 2004). It

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3 was also shown that magnesium (Pinson-Gadais et al., 2008), hydrogen peroxide (Ponts et al.,  
4 2007), temperature (Schmidt-Heydt et al., 2008) and ferulic acids (Boutigny et al., 2009)  
5 modulated *TRI* genes expression and trichothecenes production by *F. graminearum* in liquid  
6 cultures. Very recently, it has been reported that a low extracellular pH is determinant for toxin  
7 production in *F. graminearum* (Gardiner et al., 2009). It has been demonstrated that the production  
8 of aflatoxin, sterigmatocystin in *Aspergillus spp.* (Keller et al., 1997), fumonisin in *Fusarium*  
9 *verticillioides* (Flaherty et al., 2003) and a large spectrum of secondary metabolites including some  
10 antibiotics and mycotoxins are influenced by the ambient pH.  
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19 Filamentous fungi and yeasts are capable of growing over a wide range of pH due to an efficient  
20 pH homeostatic mechanism which tailors the gene expression to the environmental pH (Penalva et  
21 al., 2008). This pH regulatory system, well described in *Aspergillus nidulans*, involves six known  
22 components encoded by *pala*, *palB*, *palC*, *palF*, *pall* (Arst and Penalva, 2003) that mediates the  
23 proteolytic activation of the zinc finger transcription factor PacC in response to the alkaline  
24 ambient pH (Diez et al., 2002), which induces the alkaline-expressed genes and represses the  
25 acidic-expressed genes (Tilburn et al., 1995). More recent studies report that the pathways  
26 responsible for many secondary metabolites biosynthesis are controlled by PacC factor in various  
27 fungi suggesting an essential role for this transcriptional regulator (Espeso and Penalva, 1996,  
28 Keller et al., 1997, Flaherty et al., 2003).  
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39 In this study we analyzed pH variation during development of *F. graminearum in vitro* and the  
40 kinetic of toxin production using the minimal synthetic medium (MS) described by Boutigny et al.  
41 (Boutigny et al., 2009). Effect of pH on toxin induction was analyzed for four different *Fusarium*  
42 strains. In order to investigate the role of the ambient pH in the regulation of trichothecenes  
43 biosynthesis, we analyzed the expression of four *FgTRI5* cluster genes *TRI5*, *TRI4*, *TRI6*, *TRI10*  
44 and *TRI101* non cluster gene in addition to the probable pH response transcription factor *PAC1*, in  
45 different pH conditions. The results suggest a determinant role of acidic growth conditions on  
46 “*FgTRI5* cluster” genes expression.  
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## 55 **Materials and methods**

56 *Strain, spores preparation, culture media and culture conditions*  
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3 The *F. graminearum* strain CBS185.32 (Centraalbureau voor Schimmelcultures, The Netherlands)  
4 was used throughout this work. It is a producer of DON and its acetylated form 15ADON. In our  
5 culture conditions, the 15ADON form was predominantly produced.. Potato Dextrose Agar (PDA)  
6 medium was used for propagating the fungus. For spores suspension preparation, fungal strains  
7 were grown in the CMC sporulation liquid medium (Cappelini and Peterson, 1965) at 25°C and  
8 150 rpm into darkness for 3 days, then filtered through mira-cloth (100 µm). The filtrate was used  
9 as a spore suspension to inoculate the media during this study.

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18 All liquid-culture experiments were performed in Petri plates (Ø 55mm) containing 8 ml of the  
19 appropriate culture medium inoculated with the appropriate spore suspension to reach a final  
20 concentration of 10<sup>4</sup> spores/ml. The culture media used in this study were: a minimal low buffered  
21 Mycotoxins Synthetic medium (MS medium) containing KH<sub>2</sub>PO<sub>4</sub> 0.5 g l<sup>-1</sup>, K<sub>2</sub>HPO<sub>4</sub> 0.6 g l<sup>-1</sup>,  
22 MgSO<sub>4</sub> 0.017 g l<sup>-1</sup>, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 1 g l<sup>-1</sup>, glucose 20 g l<sup>-1</sup> and 0.1 ml l<sup>-1</sup> Vogel's trace elements stock  
23 solution (Vogel, 1956). This medium was already used in previous works (Boutigny et al., 2009).  
24 Its initial pH is 6.5 and quickly decreases during growth of the fungus. To obtain the buffered  
25 media, exactly the same components than the MS medium were used and buffered with a mixture  
26 of 50 mM of 3-(N-morpholino) propanesulfonic acid (MOPS) and 55 mM of 2-(N-morpholino)  
27 ethanesulfonic acid (MES) to obtain the BMS6.5 medium (MS buffered to pH6.5) or with citric  
28 acid 40 mM and Na<sub>2</sub>HPO<sub>4</sub> 20 mM to obtain the BMS3 (MS buffered to pH3). Incubation was  
29 performed at 25°C in darkness conditions without shaking. For transfer experiments, the mycelia  
30 were incubated for 3 days in the BMS6.5 medium, then collected, washed with BMS3 medium and  
31 transferred to Petri dishes containing BMS3 medium. The cultures were collected by centrifugation  
32 in sterile conditions; the liquid medium was stored at -20°C until extraction of the toxin and the  
33 mycelium was used to perform total RNA extraction or lyophilized for fungal biomass  
34 quantification either directly or after storage at -80°C. All cultures were done in triplicate.

#### 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 *Toxin analyses and pH measurements*

50 Ethyl acetate extractions of liquid culture samples were performed as previously described  
51 (Boutigny et al. 2009). Quantification was done by comparing the samples to the standard  
52 solutions of DON and 15ADON prepared from commercial pure powder samples (Sigma-Aldrich  
53 Co.) using HPLC as previously described (Bily et al., 2004). The pH of the cultures was measured  
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3 directly from the supernatants using “InoLab pH 720” pH meter following the manufacturer’s  
4 instructions (WTW GmbH, Weilheim, Germany).  
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#### 8 9 *Total RNA extraction and cDNA preparation*

10 To perform total RNA extraction, 100 mg of mycelium was ground for 1 min in 1 mL of TRIzol®  
11 reagent (Invitrogen) with the TissueLyser System® (Qiagen). Total RNA was extracted according  
12 to the manufacturer’s instructions. The quality of the RNA preparation was verified by agarose gel  
13 electrophoresis and the RNA concentration was measured using a nanodrop® ND 1000  
14 spectrophotometer (Labtech International LTD®). The reverse transcription reactions were  
15 performed on 5 µg of total RNA using the SuperScript™ II First-Strand Synthesis System for RT-  
16 PCR (Invitrogen), the thermal cycling conditions were programmed using an iCycler™ (Bio-Rad)  
17 following the manufacturer’s instructions (Invitrogen).  
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#### 26 27 *Expression analysis by real-time PCR*

28 Abundances of the transcripts of *TRI5*, *TRI4*, *TRI6*, *TRI10*, *TRI101* and *PAC1* were evaluated  
29 using a LightCycler® LC2.0 system and the LightCycler® Software 3.5.3 (Roche), with 1 µL of  
30 diluted cDNA in a 10 µL PCR reaction, using the QuantiTect™ SYBR® Green PCR Kit (Qiagen)  
31 according to the manufacturer’s instructions. Expression of *GAPDH* (Glyceraldehyde phosphate  
32 dehydrogenase) was used as endogenous control. PCR reactions were carried out at 94°C for 15  
33 min, followed by 50 cycles of 94°C for 10 s, 15 s at appropriate hybridization temperature for each  
34 primer pairs (Table 1), and 72°C for 25 s. Results were obtained from three replicate assays.  
35 Primer pairs for amplifying the *TRI* genes have been previously described (Boutigny et al., 2009)  
36 and are listed in table 1. The *GAPDH* primer pair was designed based on the sequence of *F.*  
37 *graminearum* PH-1 strain *GAPDH* (Table 1). Primers for *PAC1* were designed using the sequence  
38 of the probable pH response transcription factor *pacC* FGSG\_12970.3  
39 ([www.broadinstitute.org/annotation/genome/fusarium\\_graminearum/](http://www.broadinstitute.org/annotation/genome/fusarium_graminearum/)). The absence of non-  
40 specific PCR amplification was checked by running melting curve and agarose gel analyses of the  
41 final PCR product.  
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3 PCR efficiencies ( $E$ ) were determined as previously described (Ponts et al., 2007) using a standard  
4 curve generated from five log dilutions of a standard cDNA mixture prepared by mixing a fraction  
5 of each cDNA samples.  
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#### 8 9 10 *Real-time PCR data analysis*

11 In order to verify the stability of *GAPDH* expression throughout the culture time course, we  
12 proceeded to the comparison of the crossing point values ( $C_p$ ). The  $C_p$  values experimentally  
13 measured for the target *TRI* genes were compared in the different pH conditions of culture and  
14 normalized to the  $C_p$  values obtained for the reference gene *GAPDH*. Then, the expression levels  
15 of the target genes, normalized by the reference gene expression were compared at different pH  
16 values using the REST® software (Relative expression Software Tool) (Pfaffl, 2001, 2002).  
17 Expression of the target genes in the neutral pH medium BMS6.5 has been always chosen as  
18 control condition and the results were given as values relative to the control in each experiment.  
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## 28 **Results**

### 29 *Pattern of fungal development, toxin production and pH variation in MS medium*

30 The main objective of this experiment was to determine the pattern of pH evolution and the  
31 kinetic of type B trichothecenes accumulation during the development of *F. graminearum*.  
32 Cultures were performed in liquid conditions without shaking in MS medium which is a low  
33 buffered poor minimal medium specifically adapted for B trichothecenes production by *Fusarium*  
34 (Boutigny et al. 2009). During the culture the *F. graminearum* CBS 185.32 strain, the growth  
35 begins to be visually detectable two days after inoculation (Figure 1). The full development  
36 stopped at day 3 with a dried mycelium mass weighting around 45 mg per 8 ml of culture medium.  
37 The production of trichothecenes starts to be detected at day 3 of culture, increases rapidly the two  
38 following days and the toxin continues to accumulate until day 16. Only the sum of DON and  
39 15ADON is reported as the two forms followed the same pattern of toxin production. pH  
40 measurement, performed throughout the culture, shows a prompt acidification two days after  
41 inoculation. The initial pH of the medium is 6.5 at inoculation and drops to 3 after only 3 days of  
42 culture. This acidification coincides with the initiation of trichothecene B production.  
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### 55 *Effect of pH change on fungal development and TCT B production*

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3 In order to study in details the effect of pH variation on toxin production and fungal development,  
4 culture were carried out in three pH conditions: First, a culture in the MS medium as above serves  
5 as the control. Secondly, a culture in the BMS6.5 “neutral” medium in which buffering capacity  
6 was increased in order to maintain the pH to 6.5. For the third condition, the fungal development  
7 was achieved for three days in the BMS6.5 medium and the mycelia were transferred to the BMS3  
8 medium buffered to maintain pH3. The pattern of the pH variation in different culture media was  
9 followed. In the control in MS medium, natural acidification occurred during the growth of  
10 mycelium leading quickly to pH 3. As expected, in the BMS6.5 medium, the pH was maintained  
11 close to neutrality throughout the 7 days of culture. The BMS3 medium buffered at pH 3  
12 maintained this very acidic pH starting with the transfer of mycelium and throughout the duration  
13 of the culture. In the buffered media, the pH maintained a value in the expected range (initial pH  $\pm$   
14 0.2 pH units) which confirms the reliability of the buffers used during this study.  
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26 The dry fungal biomass was determined (Figure 2A). The overall development of the mycelium in  
27 the three conditions was maximal at day 3 of culture. In the MS medium, where the acidification  
28 occurred naturally during the fungal development, the biomass accumulation is lower than in the  
29 BMS6.5 in which the natural acidification is prevented by the buffer. In the third culture condition,  
30 where the mycelium accumulated until 3 days at pH 6.5 and was then transferred to medium  
31 buffered at pH 3, the biomass did not increase after the transfer.  
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39 The type B trichothecenes accumulating in the culture medium were quantified by HPLC-DAD  
40 (Figure 2B). In the MS medium, the toxin production starts to be detectable at day 4 and  
41 accumulates the following days. In the BMS6.5 medium, no toxin at all is detected throughout the  
42 culture course. However, in the third culture condition (transfer to the acidic condition), the toxin  
43 production is induced but it begins to be detectable only at day 5 and significantly increase at day  
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#### 51 *TRI genes expression is induced by acidic pH*

52 In order to investigate whether or not the regulation of toxin biosynthesis by the ambient pH is due  
53 to a molecular regulation of the “*FgTRI5*” cluster, expression of *TRI5*, *TRI4*, *TRI6*, *TRI10*,  
54 *TRI101* and *PAC1* genes was analyzed by Q-RT-PCR. Using the three pH conditions detailed  
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3 above, the expression levels were compared at day five. As *GAPDH* gene maintains a relatively  
4 stable expression during the culture and in the different conditions, its level of expression served as  
5 control and was used to normalize *TRI* genes expression (Figure 3). *TRI5* and *TRI4* encoding the  
6 enzymes implicated in the first steps of the biosynthetic pathway are highly expressed in the acidic  
7 media (MS and BMS3 media) compared to “neutral” medium (BMS6.5) in which their expression  
8 is residual. The expression of *TRI6* and *TRI10*, encoding the regulators of the other *TRI* genes, are  
9 also induced and their level of expression in the “acidic” media can be largely sufficient to explain  
10 the strong activation of *TRI5* and *TRI4*. The *TRI101* gene is interesting because it encodes an  
11 enzyme involved in an intermediate step of the biosynthesis pathway but it is located outside the  
12 “*FgTRI5* cluster” (Kimura et al. 2007). As other *TRI* genes, this gene also shows an activation of  
13 expression. However, its differential of expression between acidic and neutral pH seems less  
14 important than what is observed for the genes located in the “*FgTRI5* cluster”. In contrary to *TRI*  
15 genes, the quantification of *PAC1* expression shows that as expected, this gene is highly expressed  
16 under neutral pH, and the acidification clearly represses its expression.  
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### 30 *TRI5* and *TRI101* respond differentially to the pH variation

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32 The expression kinetic of *TRI5* and *TRI101* was compared from the third day to the seventh day of  
33 culture. Histograms (Figure 4) show their relative expression in the “acidic” media compared to  
34 their expression in the “neutral” medium (BMS6.5). Expression of *PAC1* was also investigated. In  
35 both media where acidification occurred either naturally or by transfer of the culture, expression of  
36 *PAC1* gene was repressed immediately in response to acidification. This repression is maintained  
37 at any time of the culture (Figure 4A,B). Similarly, *TRI5* expression showed an immediate  
38 response to the acidification. At day 3, *TRI5* was highly expressed in the MS medium in which the  
39 pH already dropped to 3 at that time (Figure 4A); its expression conserved the same level in the  
40 following days. In opposite, the *TRI101* gene responded more progressively to the pH variation  
41 and still increased at the seventh day of culture. Similarly, when the mycelium was transferred  
42 from neutral to acidic pH (BMS6.5 → BMS3) at day 3, *TRI5* showed an immediate response to the  
43 pH shift as a high level of expression was observed one day after the transfer (day 4 of culture).  
44 Again, the *TRI101* gene showed a low level of expression at day 4 with progressive increase the  
45 following days (Figure 4B). This suggests that the two *TRI* genes respond differentially to the  
46 induction by acidic pH.  
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*Trichothecene B induction by acidic pH is common to different Fusarium strains and species*

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In order to verify whether the effect of pH change observed here is not a particularity of the *F. graminearum* CBS 185.32 strain chosen for this study, the effect of pH variation on other strains of *Fusarium* was analyzed. Strains of different chemotype (i.e. producing different type B trichothecene) were selected in our laboratory collection. The same three culture conditions as used above were applied to four *Fusarium* strains either from the *graminearum* or the *culmorum* species. Toxin measurements at day 7 showed results very similar to the one observed for *F. graminearum* CBS185.32 strain since all the four tested strains produced high amount of trichothecenes under acidic but never produced any toxin under the neutral pH growth conditions (Figure 5 in supplementary section).

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**Discussion**

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The minimal synthetic medium used during this study is low buffered, thus the observed acidification is suspected to be the result of ammonium consumption during fungal development (Jernejc and Legisa, 2001). The shutdown in the fungal development, concomitant with the acidification, can be explained by the stressing conditions due to the extreme pH although the exhaustion of one of the nutriment sources (ammonium or glucose) cannot be excluded. Control cultures in GYEP medium which contains low ammonium concentration also showed an acidification during fungal development. However, the extent of the pH drop was always greater in the MS medium where the level of toxin production was largely higher (data not shown). This may suggest that the strong acidification promotes the high level of toxin production, although, the fungal development is optimal only under neutral pH. Also, the results presented here clearly demonstrate that preventing acidification by maintaining a neutral pH in the medium prevents the induction of toxin biosynthesis. An artificial acidification conducted by transferring the mycelium from neutral (BMS6.5) to acidic pH (BMS3), leads finally to accumulation of the toxin, suggesting that low pH is necessary for the induction of trichothecenes biosynthesis *in vitro*.

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Our results showed that the expression of *TRI5* gene seems to be directly induced by acidic pH meanwhile *TRI101* expression seems to be more related to the level of trichothecene accumulation.

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3 This is coherent with the fact that *TRI101* gene is not located in the “*FgTRI5* cluster” and with the  
4 role of self-protection assigned to the *TRI101* gene product (Kimura et al. 1998) whose expression  
5 could be related to the quantity of toxin produced and suggests a feedback regulation. However  
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7 *TRI101* expression has recently been shown to strongly depend of *TRI6* gene expression in *F.*  
8 *graminearum* and was not increased by trichodiene supplementation in a *TRI5* deletion mutant  
9 (Seong et al. 2009). Thus, *TRI5* and *TRI101* expression levels are regulated differently one another  
10 while both are under *TRI6* control (Peplow et al. 2003). This observation may indicate that *TRI6*  
11 and *TRI10* are not the only factors that control the *TRI* genes expression. Our results suggest that  
12 the mechanism of regulation by the ambient pH mediating the regulation of TCT B production acts  
13 through the strong modulation of various *TRI* genes expression, especially those located in the core  
14 “*FgTRI5* cluster”.

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25 Taken together, the data presented here are in agreement with the recently published study  
26 (Gardiner *et al.* 2009) where it has been showed that the highest level of *TRI5* expression and  
27 DON accumulation is reached under pH 3 and it reduces drastically as far as the pH progress to  
28 neutrality. Although the induction of *TRI5* in response to low pH was previously reported  
29 (Gardiner *et al.* 2009), this study elucidate for the first time, the differential pattern of *TRI* genes  
30 expression in response to the ambient pH. Also, it brings forward that the regulation of  
31 trichothecenes B production by the ambient pH is a general mechanism in *Fusarium species*  
32 producing trichothecenes, independently from strains and toxin chemotype.

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41 Acidification seems to be mandatory for toxin production and *TRI* genes expression *in vitro* in *F.*  
42 *graminearum*. This main conclusion supports the hypothesis that trichothecene biosynthesis could  
43 be negatively regulated by the general pH regulatory system. In *Aspergillus*, the dissection of the  
44 pH regulatory system common for most fungi revealed the presence of PacC, a zinc finger  
45 transcription factor able to activate alkaline expressed genes and repress acidic expressed genes by  
46 binding to “GCCARG” consensus sequences located in their promoters. PacC itself is activated  
47 under neutral or alkaline pH by the Pal signalling pathway (Arst and Peñalva, 2003). Orthologues  
48 of all these components of the pH regulatory system are present in the genome of *F. graminearum*  
49 and the “GCCARG” consensus binding sequences is detected in various genes promoters of the  
50 “*FgTRI5* cluster” (data not shown). In addition we showed that expression of the probable  
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*FgPAC1* gene is high when *TRI* genes are not expressed and is always repressed when *TRI* genes are induced. Whether this *F. graminearum* potential homologue of PacC really represses *TRI* genes expression in this fungus is now under investigation in our laboratory using molecular approaches.

## Acknowledgments

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Table 1. Primer pairs used to amplify *GAPDH*, *TRI5*, *TRI4*, *TRI6*, *TRI10*, *TRI101* and *PAC1* cDNA by Real-Time PCR and PCR efficiencies obtained for five log dilutions of the cDNA mix.

Gene	Accession No.	Forward primer sequence (5' to 3')	Reverse primer sequence (5' to 3')	T <sub>m</sub> (°C)	PCR efficiency
<i>GAPDH</i>	FG06257	CCTTTCATTGAGCCTCAC	CGTACATGGGAGCGTC	59	2
<i>TRI5</i>	FG03537	GACCCTAAGCGACTACAG	GTGCTACGGATAAGGTTTC	58	1,99
<i>TRI4</i>	FG03535	TATTGTTGGCTACCCCAAGG	TGTCAGATGCGCCTTACAAA	58	1,96
<i>TRI6</i>	FG03536	AGCGCCTTGCCCTCTTTG	AGCCTTTGGTGCCGACTTCTTG	58	1,96
<i>TRI10</i>	FG03538	TCTGAACAGGCGATGGTATGGA	CTGCGGCGAGTGAGTTTGACA	58	1,93
<i>TRI101</i>	AB011417	AGGGCATTAGCGAGGGAAACACA	ACGGCACGCCTTGGAGAGTAGAC	59	1,97
<i>PAC1</i>	FGSG12970.3	GAGCCAGGCATGCGAAGAATG	CTCTGGAGGTATGCTTCAGCGG	66	1.94

Figure 1. Kinetics of *F. graminearum* CBS185.32 growth, toxin production and pH change in MS liquid medium. Inoculation was done at day 0 and cultures were stopped at nine different times (One, two, three, four, six, eight, 10, 13, 16 days after inoculation). Fungal biomass (mg per culture) and toxin measurements (sum of DON + 15ADON) are the result of three biological replicates. Error bars are the standard error of the mean.

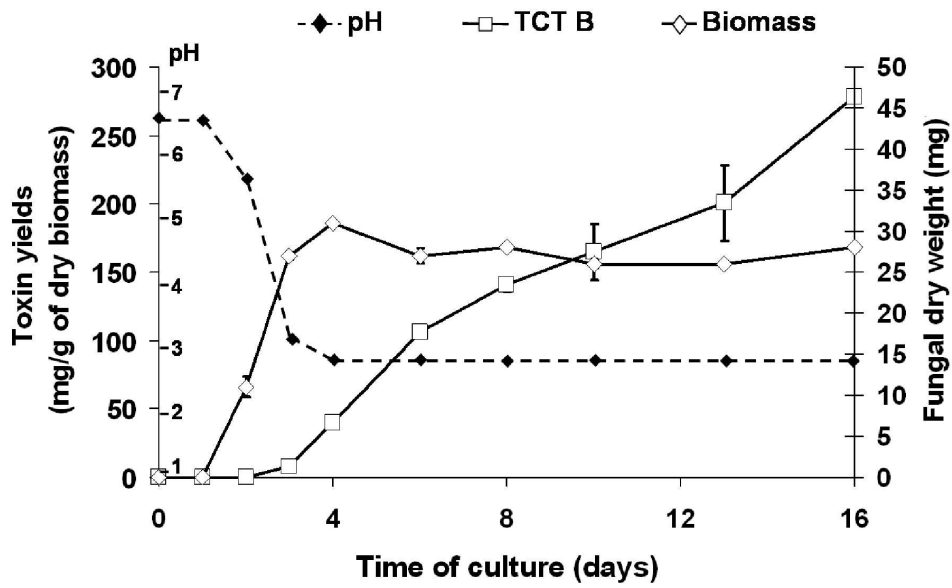
Figure 2. Effect of pH on toxin production and fungal growth. Kinetic of fungal biomass (A) and trichothecenes B production (DON + 15ADON) (B) during culture in three different culture conditions: (i) MSM: MS medium (low buffered); (ii) BMS6.5: MS medium buffered to pH6.5 using MOPS/MES buffer and (iii) BMS6.5 → BMS3: Culture was done in BMS6.5 medium then

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3 transferred at day 3 to BMS3 medium buffered at pH3. The cultures were stopped at day 3, 4, 5  
4 and 7 days after inoculation. nd: non detectable. Values are the result of three biological replicates.  
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6 Error bars are the standard error of the mean.  
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16 Figure 3. Effect of acidic pH on *PAC1* and *TRI* genes expression. The histograms represent (in  
17 logarithmic (log<sub>2</sub>) scale) the expression ratio of *TRI5*, *TRI4*, *TRI6*, *TRI10*, *TRI101* and *PAC1* at day  
18 5 in the MS medium (white bars) and in the BMS3 medium two days after transfer from BMS6.5  
19 (dashed bars) compared to their expression in BMS6.5 medium.  
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28 Figure 4. Kinetic of *TRI5*, *TRI101* and *PAC1* expression in response to pH change. Histograms  
29 represent the expression ratio of *TRI5* (white bars), *TRI101* (dashed bars) and *PAC1* (black bars) in  
30 MS medium (A) and BMS3 medium (B) relative to the expression in BMS6.5 medium after three,  
31 four, five and seven days of culture. For the BMS3 medium (B), the transfer from BMS6.5 was  
32 done at day three, so genes expression for the two compared conditions was considered similar  
33 (ratio=1) for this day, thus the value is zero in the logarithmic scale.  
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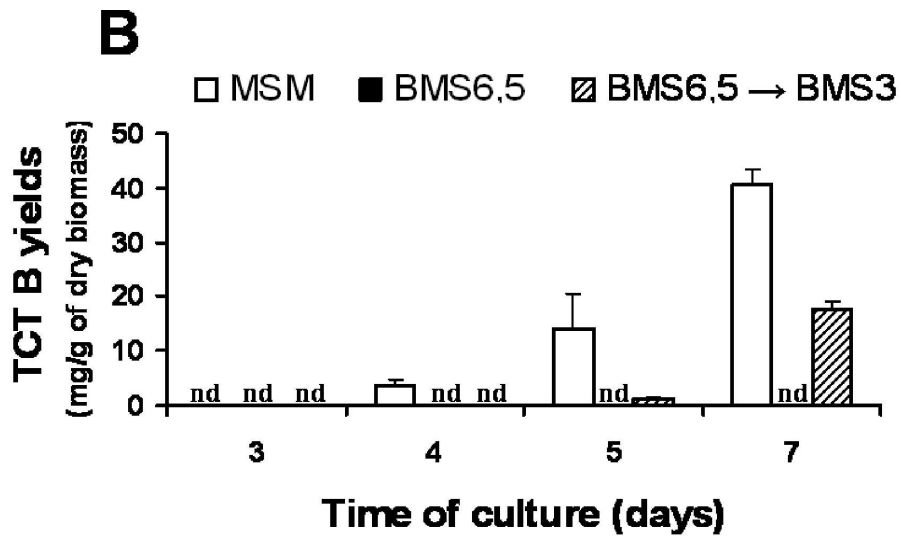
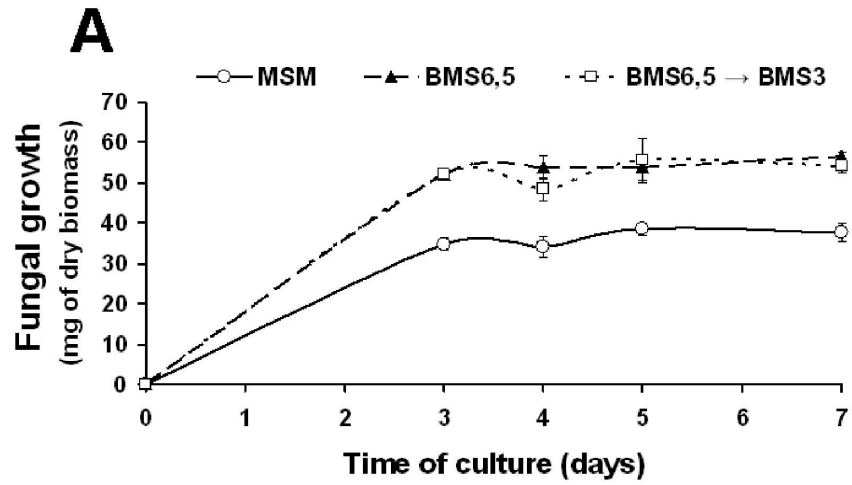
44 Figure 5. The behaviour of TCTB production in different *Fusarium spp.* strains after pH change.  
45 Type B trichothecenes (DON + ADON or NIV + 4ANIV) accumulation after 7 days of culture in  
46 three different culture conditions: MSM: (i) MS medium (low buffered); (ii) BMS6.5: MS medium  
47 buffered to pH6.5 using MOPS/MES buffer (toxin not detected) and (iii) BMS6.5→BMS3:  
48 Culture was done in BMS6.5 medium than transferred at day 3 to BMS3 medium buffered at pH3.  
49 The tested strains are *F. culmorum* INRA 233 (DON/15ADON), *F. culmorum* INRA 117  
50 (DON/3ADON), *F. graminearum* INRA 155 (DON) and *F. culmorum* INRA 319 (NIV/4ANIV).  
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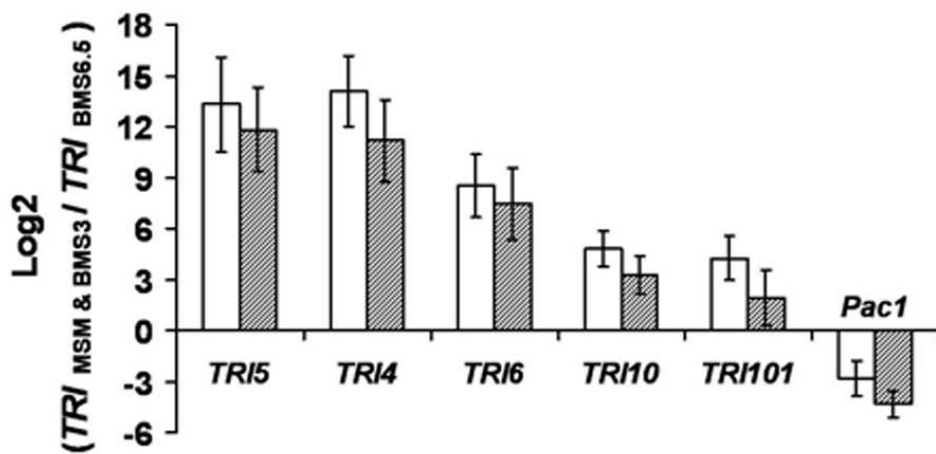
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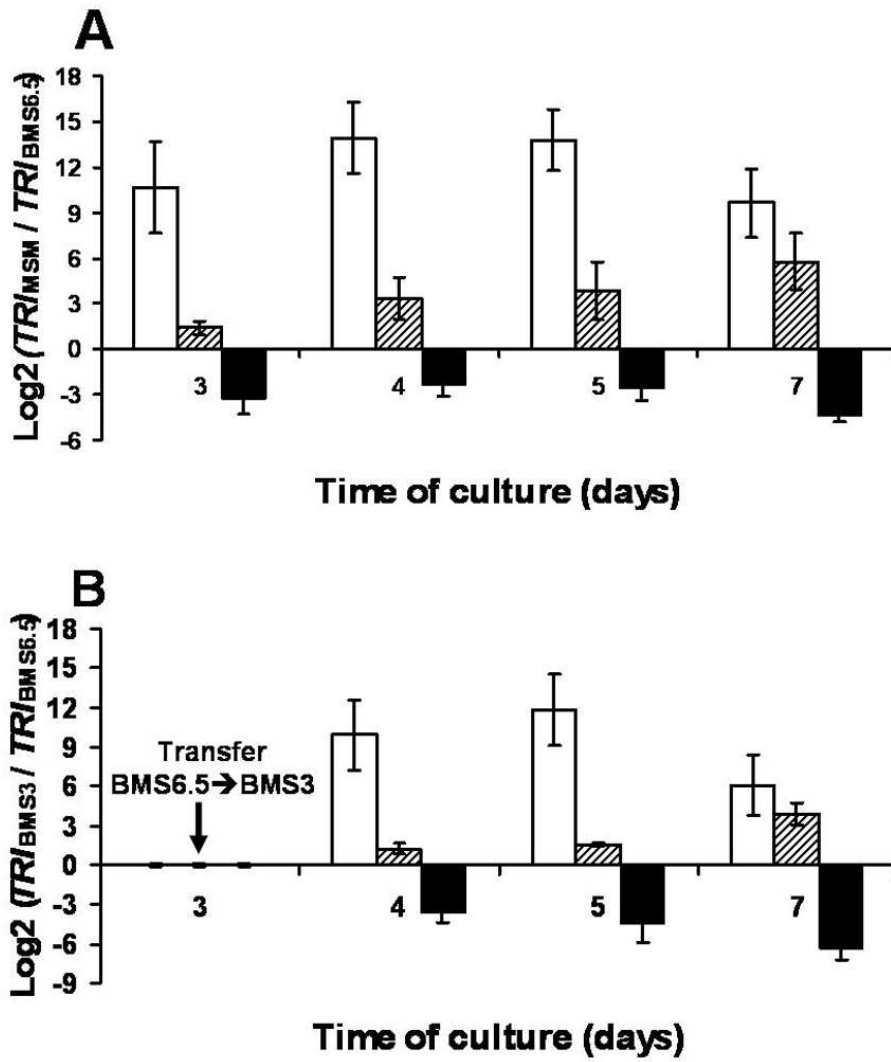
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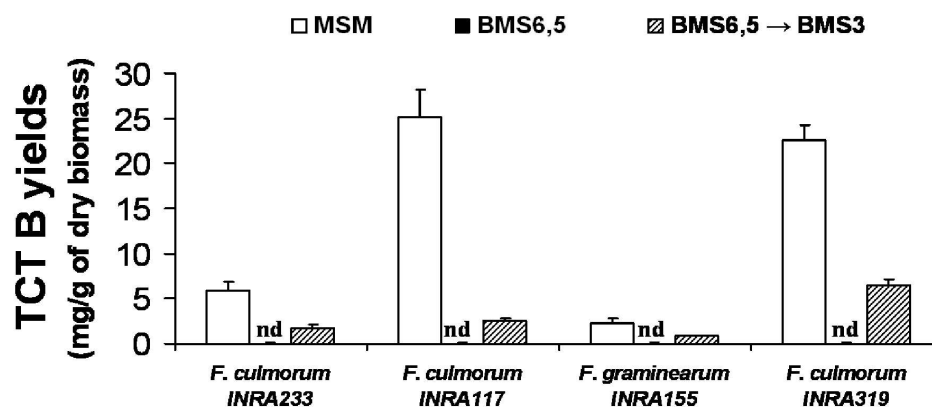
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Supplementary section: The behaviour of TCTB production in different *Fusarium* spp. strains after pH change. Type B trichothecenes (DON + ADON or NIV + 4ANIV) accumulation after 7 days of culture in three different culture conditions: MSM: (i) MS medium (low buffered); (ii) BMS6.5: MS medium buffered to pH6.5 using MOPS/MES buffer (toxin not detected) and (iii) BMS6.5→BMS3: Culture was done in BMS6.5 medium than transferred at day 3 to BMS3 medium buffered at pH3.

The tested strains are *F. culmorum* INRA 233 (DON/15ADON), *F. culmorum* INRA 117 (DON/3ADON), *F. graminearum* INRA 155 (DON) and *F. culmorum* INRA 319 (NIV/4ANIV). nd: non detectable.

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