Toxigenic capacity and trichothecene production by *Fusarium graminearum* isolates from Argentina and their relationship with aggressiveness and fungal expansion in the wheat spike

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ABSTRACT

At least 20 epidemics of Fusarium head blight (FHB) of wheat have been registered in the last 50 years in Argentina, with variable intensity. Damage induced by the disease is further aggravated by the presence of mycotoxins in affected grains that may cause health problems to human and animals. The trichothecene chemotype was analyzed for 112 isolates of *Fusarium graminearum* from Argentina by polymerase chain reaction and two field trials were conducted to study the aggressiveness of a sub-sample of 14 representative isolates and to analyze deoxynivalenol (DON) production *in planta* and *in vitro*. All isolates belonged to the 15-ADON chemotype. Significative differences were observed in both the symptom severity induced in wheat spikes and the *in vivo*

DON production, and a close correlation was found between these two variables. However, *in vitro* toxigenic potential was not correlated with the capacity of *F. graminearum* isolates to produce DON under natural conditions. The progress of infection in the rachis of inoculated wheat spikes was analyzed and the pathogen presence verified in both symptomatic and symptomless spikes. Even isolates with a limited capacity to induce symptoms were able to colonize the vascular tissue and to produce considerable amounts of DON *in planta*.

1. INTRODUCTION

Fusarium head blight (FHB) of wheat, caused by *Fusarium graminearum* (Schwabe), is a destructive disease that frequently causes epidemics in many wheat cropping areas worldwide (15,39). In Argentina, at least 20 epidemics have been registered in the last 50 years with variable intensity, the more severe occurring in 1945-46, 1976, 1978, 1985, 1993 and 2001 (20,29,44).

The disease affects several major crops, including maize, wheat, barley, rye and triticale, reducing seed and grain quality as well as yield, leading to poor germination and reduction in flour baking properties. The damages induced by the disease are further aggravated by the frequent presence of mycotoxins in affected grains. These persistent, thermostabile metabolites, produced in association with food and feeds, may cause health problems to human and animals even in low doses (28).

The trichothecenes deoxynivalenol (DON), its derivatives 3-acetyl-DON (3-ADON) and 15-acetyl-DON (15-ADON), and nivalenol (NIV) and its acetylate derivative Fusarenone X (FUS-X) are the major mycotoxins produced by *F. graminearum* (37).

Deoxynivalenol, the most important mycotoxin in terms of human exposition (43), has been found as a contaminant of wheat, maize and barley, three crops that account for two thirds of the worldwide production of cereals.

Trichothecenes are synthesized by a complex metabolic route that involves a series of oxygenations, isomerizations and esterifications (3). According to the pattern of trichothecene production, isolates of *F. graminearum* can be organized in three different chemotypes: *Ia*, producing DON and 3-ADON; *Ib*, producing DON and 15-ADON¸ and *II*, producing NIV and FUS-X (25,45). Most of the genes involved in the biosynthesis of trichothecenes, and thus in its variation, are localized in a cluster of at least 10 genes (8) that encode regulatory proteins as well as most of the enzymes required for trichothecene synthesis (3). Among them, *Tri13* and *Tri7* are responsible for the production of DON- or NIV-like trichothecenes (31), while differences in the sequence of *Tri8* have been identified as the basis for the production of either 3-ADON or 15-ADON (2). The identification of these genes provided the basis for the development of valuable polymerase chain reaction (PCR) tools for fast chemotype determination in *F. graminearum* (59,63).

Differences in the geographic distribution of *F. graminearum* chemotypes have been reported. In Chinese populations of the pathogen, DON-like chemotypes prevail, with similar frequencies of 15-ADON and 3-ADON strains (65), while in Japan the prevalent chemotype is NIV (60). In Europe 15-ADON is the most frequent chemotype, followed by 3-ADON and only a few representatives of NIV strains (27,48,52). In USA and Canada, where 15-ADON was previously identified as the predominant chemotype, increasing frequencies of 3-ADON and NIV strains have been recently reported (53,59).

In Brazil and Uruguay, 15-ADON is the most commonly found trichothecene chemotype with only sporadic recovery of strains of NIV chemotype (7,47).

Controversy exists regarding the chemotype composition of *F. graminearum* populations from Argentina. Several authors reported the prevalence of DON chemotypes in the wheat cropping area (4,10,17,55), while others reported also the presence of NIV chemotypes (19,34,56,58). These antecedents suggest that, even when DON seems to be the predominant chemotype in *F. graminearum* isolates from Argentina, the potential exists for the occurrence of grain contamination with NIV.

Qualitative and quantitative differences in the synthesis of enzymes and mycotoxins might be responsible for the great variability in aggressiveness observed within *F. graminearum* populations (42). The phytotoxic activity of trichothecenes, and particularly of DON, has been widely studied. On plant tissue, these compounds inhibit protein synthesis and mitochondrial function and affect cell division and membranes (57). Reduced production of trichothecenes does not influence the capacity of *F. graminearum* to infect wheat or maize, but affects the progression of the infection due to a decrease in aggressiveness (50,51). However, the mechanism by which these metabolites induce the development of FHB remains largely unclear. It has been proposed that during disease spread, trichothecenes could be translocated in the plant before the growth of the pathogen, reducing protein synthesis without inducing the activation of defense mechanisms (46,49). Furthermore, trichothecenes have been found to inhibit the development of defense mechanisms in the wheat rachis node that would otherwise prevent the movement of the fungus into the internode (26). On the other hand, evidence has been found that DON may activate a range of plant defense responses and programmed cell death in wheat, simultaneously favoring necrotrophic growth of the pathogen and stimulating antimicrobial defense responses in the host (14).

A correlation between aggressiveness and DON production has been proposed for *F. graminearum* and *F. culmorum* (13,62). McCormick (37) suggested that the role of trichothecenes as aggressiveness factors highlights the possibility of developing wheat cultivars resistant to the accumulation of mycotoxins that could improve plant response to the disease. However, experiments on wheat have yielded controversial results and several authors have either failed to find a significative correlation between FHB and production of trichothecenes or found inconsistent results (1,6,33,61).

In Argentina, a high level of variability in the aggressiveness of *F. graminearum* was found within isolates from wheat (5,36). Alvarez et al. (5) carried out specific assays but were unable to establish the relationship between FHB symptoms on wheat spikes, toxigenic capacity of *F. graminearum* isolates and progression of the pathogen on wheat tissue.

Therefore, the clarification of chemotype distribution and of the relationship between mycotoxin accumulation and aggressiveness of isolates of *F. graminearum* from Argentina remains an issue of great importance, since it could greatly help the development of wheat genotypes tolerant or resistant to FHB. With this in mind, the objectives of the present work were: a) to identify the mycotoxin chemotypes of *F. graminearum* isolates from the Buenos Aires Province in Argentina; b) to quantify their toxigenic capacity and c) to correlate the accumulation of trichothecenes in wheat grains with aggressiveness of the pathogen and its capacity to colonize the spike.

2. MATERIALS AND METHODS

2.1. *Fusarium graminearum* chemotype analysis

Chemotype analysis were carried out on 112 *F. graminearum* isolates obtained from grain samples of common wheat (*Triticum aestivum* L.) collected from 28 different localities distributed within Buenos Aires Province, Argentina (36). Isolate chemotypes were determined by means of the two multiplex PCRs developed by Starkey et al. (59) using the chemotype-specific primers previously validated by Ward et al. (63). The sequence of the primers used, target genes, and amplified products are detailed in Table 1.

Both multiplex-PCRs were carried out in a 25 µL volume containing 5-10 ng of genomic DNA, 1 U of T-plus DNA polymerase (Highway Molecular Biology- InBio-UNICEN, Argentina), 0.5 µM of each primer (FAGOS/Ruralex, Argentina), 200 µM of each deoxynucleoside triphosphate and 1.5 mM $MgCl_2$ in 1x reaction buffer (500 mM KCl, 100 mM Tris-Cl pH 9.0, 1% Triton X-100 without Mg^{++}). Both PCR cycles consisted of an initial denaturation step of 3 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 53°C, 1 min at 72°C and a final extension step of 10 min at 72°C. Amplification products were resolved on 1.5% (w/v) agarose gels containing 0.2 mg mL^{-1} ethidium bromide in tris-borate-EDTA buffer. Gels were visualized under UV using a GeneGenius (Syngene, USA) image analyzer. Photos were taken with GeneSnaps software and the size of the resulting fragments was estimated using GeneTools and GeneDirectory software by comparison with the bands generated by a 1 kb DNA ladder (Highway Molecular Biology- InBio- UNICEN, Argentina). In each of the amplification reactions a negative control without DNA and positives controls for each chemotype were included.

2.2. Evaluation of the aggressiveness of *Fusarium graminearum* isolates

Two field trials were conducted in consecutive years to evaluate aggressiveness of the 112 isolates of *F. graminearum* using common wheat cv. Klein Chajá (36). In both trials 20 field grown wheat spikes were point-inoculated with each isolate at anthesis (Zadoks growth stage 65; (64)) in a completely randomized block design with four replicates, each consisting of five spikes.

Spikes were visually rated for disease severity 21 days post-inoculation (dpi) considering symptomatic spikelets for each spike above and below the point of inoculation (PI). Disease severity, thousand kernel weight (TWK) and area under the disease progress curve (AUDPC) differences between isolates, as well as the correlations found between these parameters are reported elsewhere (36). The spikes inoculated with 14 isolates were selected to further evaluate infection progress along the rachis and mycotoxin production. These isolates were selected because they reflected the variability present among the aggressiveness groups constructed with the results obtained from the field tests (36).

2.3. Analysis of the wheat spike colonization

At maturity, the wheat spikes inoculated with each one of the 14 selected *F. graminearum* isolates in each of the two field tests were hand-threshed. The grain was saved for TKW and mycotoxin determinations while the resulting rachis were superficially disinfected by dipping in 70° ethanol for 1 min and in a 5% commercial NaClO solution (55 g of Cl L^{-1}) for 1 min, followed by rinsing in distilled water for 5 min. Surface-sterilized rachis were cut, so that each of the resulting fragments consisted of a rachis node and the adjacent internode, and plated on potato dextrose agar medium (PDA) 2% (w/v) supplemented with 250 mg L^{-1} of chloramphenicol and 600 mg L^{-1} of pentachloronitrobenzene (PCNB 75% wettable powder). Fragments were numbered with positive or negative successive numbers according to their relative position from the PI and the disposition of the fragments of the rachis in the spike was taken into consideration when plating (Figure 1). The rachis of the control treatments of the field tests were used as a control.

After 5 days of incubation at 25°C ± 2°C, expansion in the rachis was evaluated as the percentage of rachis fragments colonized by *F. graminearum*. To avoid false positives, infection of the fragments by *F. graminearum* was confirmed by two complementary approaches: microscopic observation of the colonies obtained on PDA and of the macroconidia obtained in carnation leaves agar (CLA) and/or PCR analysis by using primers FG16N F/R, as previously reported (36).

2.4. *In vivo* and *in vitro* deoxynivalenol production

In vivo and *in vitro* DON productions were evaluated for the 14 isolates of *F. graminearum* tested for pathogen expansion along the rachis. Toxin was quantified by means of the RIDASCREEN® FAST DON (R-Biopharm, Germany) Enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer instructions (23,24). For *in vivo* DON production analysis, the grains obtained from the field test-inoculated spikes were ground, weighted and used for quantification. The grains obtained from field test control spikes were considered as negative controls.

For *in vitro* DON quantification, the 14 *F. graminearum* isolates were cultivated in triplicates in 250 mL Erlenmeyer flasks containing 25 g of polished rice and 20 g of distilled water (80% humidity). Previously autoclaved substrate was inoculated with a 5 mm diameter plug of a 7-day old *F. graminearum* colony grown on PDA. As a negative control, 3 Erlenmeyer flasks containing the rice substrate were inoculated with a 5 mm diameter plug of autoclaved PDA. Flasks were cultivated for 28 days at 25°C ± 2°C in the dark, dried in a stove with forced air circulation until constant weight, grounded and weighted.

For DON quantification, the full amount of the ground *F. graminearum*-contaminated grain from each inoculated block of both field trials was used for *in vivo* production while all the rice substrate from each Erlenmeyer flask was used for *in vitro* production. Grounded material was diluted 1:20 (w/v) in distilled water and homogenized in a magnetic stirrer for 3 min, the extract obtained was filtered through Whatman n.1 filter paper and a 50 μL aliquot was pipetted into each well of the ELISA plate for the analysis. Absorbance was measured in a Beckman Coulter dtx-800 multimode detector (Beckman Coulter, USA) at 450 nm and data were converted to DON concentration by means of the RIDASOFT Win® software using the standard curve obtained from the DON standards provided in the kit.

2.5. Statistical analysis

Data taken as percentage were arcsin-transformed prior to analysis. Aggressiveness, trichothecene production and expansion of *F. graminearum* along the spike were analyzed by analysis of variance (ANOVA) and means were compared using Tukey's test ($P \leq 0.05$). Correlations were carried out for all combinations of parameters at $\alpha = 0.05$. For *in vivo* DON production, TKW and premature bleaching of the spike, data were collected using blocks as experimental units. All the analyses were performed using Statistix v.8.

A combined analysis model (38) was applied to the disease severity at 21 dpi, phenotypic variance components were estimated from the expected mean squares and broad-sense heritability was calculated using the formula by Fehr (18) as previously reported (36).

3. RESULTS

3.1. Chemotype analysis of *Fusarium graminearum* isolates

All the 112 *F. graminearum* isolates obtained from 28 localities in Argentina belonged to the 15-ADON chemotype according to the size of the fragments amplified by both multiplex PCR protocols (59).

3.2. Selection of isolates for the evaluation of the expansion of *Fusarium graminearum* in the spike, *in vivo* and *in vitro* DON production

The 14 isolates selected were tested to check that this sub-sample reflected the results previously obtained (36). To do so, the analysis of severity 21 dpi, TKW reduction and heritability according to Fehr (18) carried out for the greater group of isolates were repeated.

The variability found in the severity of the symptoms induced in wheat spikes ($F = 7.34$; $p < 0.01$) and in the TKW relative to the uninoculated control ($F = 5.31$; $p < 0.01$) among the 14 isolates reflected the differences previously found for 112 isolates, as did the percentage of inoculated spikes showing symptoms of premature bleaching (30%). Similarly, a close correlation was found between FHB severity and the relative TKW ($r = -0.9003$; $p < 0.01$).

The heritability of symptom severity for the sub-sample was of $H^2 = 0.96$, hence very similar to the value found for the full sample. This result suggests that the greatest part of the variability found was the result of genotypic differences between the isolates rather than a consequence of the effect of the year of testing.

The results obtained in both years of field tests for severity, reduction of TKW, mycotoxin production and colonization of the rachis were pooled and values are presented in Table 2.

3.3. Progression of *Fusarium graminearum* in wheat spikes from the point of inoculation

In more than 90% of the analyzed rachis, the presence of *F. graminearum* was found colonizing the fragments of the rachis that supported the inoculated spikelets in the field tests (+0 and -0) (Fig. 2 and 3). Isolates differed significantly in their ability to colonize these fragments ($F = 7.22$; $p < 0.01$), with values that ranged from 48% to 86% of the total fragments for isolates MR18 and BA14 and SP1, respectively (Table 2).

For all the treatments and all the spikelet/supporting fragment pairs, the percentage of the presence of the pathogen in the rachis was higher than the percentage of symptomatic spikes in field tests (Fig. 2). The percentage of colonization resulted even higher than the percentage of spikes showing symptoms of premature bleaching for all of the spikelet-rachis fragment pairs, except for the most distal position (+6).

Even when no inoculated spike showed FHB symptoms above the +3 spikelet, *F. graminearum* was found colonizing the apical fragment of the rachis in more than 25% of the considered spikes.

The tendency of the distribution of FHB symptoms prevalently downwards from the PI observed in the field tests was also verified when the expansion of *F. graminearum* was analyzed (Fig. 3). The higher percentages of colonization were found for the portions corresponding to the inoculated spikelets, disregarding their relative position (-0 and +0). No significative differences were found between these portions and those located immediately above (+1 and +2) and below (-1 and -2) them. The downwards distribution of the pathogen was verified from the +3 and -3 fragments onwards, being the fall of the percentage of rachis fragments colonized much more pronounced upwards than downwards the PI, with final values of over 75% and 25% for the -6 and +6 fragments, respectively (Fig. 3).

The percentage of colonization of the rachis fragments by *F. graminearum* was significantly correlated with the severity of FHB symptoms ($r = 0.7106$; $p < 0.01$) and, to a lesser extent, with the relative TKW ($r = -0.6413$; $p < 0.05$).

3.4. *In vivo* and *in vitro* deoxynivalenol production

Isolates differed in their toxigenic potential (Table 2). Significant differences existed for *in vivo* DON production when the grains collected from the spikes tested in the field were analyzed by means of ELISA ($F = 5.91$; $p < 0.01$). Toxin production ranged from 4.5 ppm for the isolate MR18 to 31.3 ppm for isolate SP1. Significant differences were also found between isolates for *in vitro* DON production on sterile rice of 80% relative humidity ($F = 4.33$; $p < 0.01$). In this case, mycotoxin accumulation values ranged from 126 ppm for isolate MR25 to 373 ppm for isolate MR46 (Table 2).

A close correlation was found between the severity of the symptoms induced by the isolates in the field tests and *in vivo* DON production ($r = 0.9073$; $p < 0.01$), as well as between pathogen colonization of the rachis and DON accumulation in the grains ($r = 0.8081$; $p < 0.01$). *In vitro* DON production and severity, on the other hand, were not significantly correlated ($r = 0.3133$), and neither were colonization of the rachis and DON production in rice substrate ($r = 0.4835$) or toxin accumulation *in vivo* and *in vitro* (r = 0.1440). The regression analysis showed a linear and positive relation between *in vivo* DON production and severity of FHB symptoms ($R^2 = 0.5683$) (Fig. 4).

4. DISCUSSION

Differences in toxicity between DON and NIV (12) make the accurate determination of the chemotypes of *F. graminearum* found in different cropping areas of the world an important factor for the prediction of the risks related to the occurrence of mycotoxin contamination in food and feed.

In Argentina, controversy exists on the prevalence of *F. graminearum* chemotypes. Most of the work carried out with Argentinian isolates of the pathogen suggests that the predominant trichothecene is DON (4,10,35,54,56); nonetheless, potential for NIV contamination could be present (19,22,34).

Here, we analyzed 112 isolates of *F. graminearum* obtained from 28 localities of the Buenos Aires Province in Argentina (36) to determine their chemotype. All tested isolates were characterized as 15-ADON producers based on the amplification of specific fragments of two trichothecene core genes using multiplex PCR (59,63). Our finding agrees with previous reports on the prevalence of DON chemotype in populations of the pathogen from wheat in Argentina (4,10,35,54,56). In maize, on the other hand, Sampietro et al. (58) reported an important presence of isolates belonging to the NIV chemotype, which the authors attributed to potential differences in the preference for the different hosts. These results suggest that the toxigenic potential present in *F. graminearum* populations from Argentina is complex and that there is a chance for significant variation in the range of mycotoxins that could potentially contaminate food and feeds.

In a previous work we reported the aggressiveness variation present in 112 isolates of *F. graminearum* obtained from 28 localities of Buenos Aires Province in Argentina following inoculation on field grown wheat spikes (36). To check the representativeness of a sub-sample of 14 of these isolates, the same parameters previously analyzed for the complete collection of isolates were taken into consideration. The results obtained indicate that the findings previously reported are also valid for these 14 isolates, and that the sub-sample chosen is representative of the behavior shown by the complete collection (36).

The present work is the first attempt to analyze and relate both the capacity of *F. graminearum* isolates from Argentina to induce symptoms in field-grown wheat spikes and DON production under both *in vitro* and *in vivo* culture conditions. Isolates differed in their ability to produce mycotoxins both in the grains obtained from field-grown wheat spikes (*in vivo*) and under laboratory conditions on a rice substrate (*in vitro*). Under field conditions, the amount of DON produced surpassed in all cases the 2 ppm tolerance limits for commercialization imposed by the European Union standards (16). *In vitro* DON production was several folds greater than *in vivo* production for all isolates, but no correlation was found between these two parameters. Similar results were obtained by Walker et al. (61) when testing DON production under field and laboratory conditions. These results suggest that the potential for mycotoxin production shown by *F. graminearum* under controlled conditions has no correlation with its capacity to produce them *in planta*.

Unlike previous results obtained with *F. graminearum* isolates from Argentina (5), a close correlation between the severity of the symptoms induced by the isolates in field tests and *in vivo* DON production is reported here. Furthermore, the regression analysis performed showed a positive linear relation between the severity of FHB and DON accumulation in the grain. These results confirm that the trichothecene contamination of wheat grains is a consequence of the severity of FHB symptoms and agree with those obtained by Mesterházy et al. (41) and Mesterházy (40).

On the other side, neither the aggressiveness of the isolates nor the relative TKW were correlated with *in vitro* toxin accumulation. Among the resistance mechanisms present in wheat genotypes towards FHB, types III (resistance to infection of the grain) and IV (tolerance to infection) could be related to the capacity of the host to degrade and/or tolerate DON (30). Recently, it has been demonstrated that some wheat lines possess the ability to detoxify DON by its glucosylation and that this ability is linked to the quantitative trait locus (QTL) *Qfhs.ndsu-3BS* (32). The presence of these or other detoxification mechanisms in the host and their interaction with aggressiveness and toxigenic capacity of *F. graminearum* isolates could be responsible for the absence of a relationship between *in vitro* and *in vivo* DON production.

The colonization of the rachis by *F. graminearum* isolates in inoculated wheat spikes is a variable trait. For all the analyzed treatments and spikelet-rachis portion pairs; the percentage of colonization was higher than the visible FHB symptoms. Furthermore, the tendency observed in the distribution of FHB symptoms in the spike from the PI downwards (36) was verified when the colonization of the rachis was considered. These results confirm that the progress of *F. graminearum* invasion along the spike mainly follows a basipetal route along the vascular tissues and agree with recent reports (9). Differences in connection between the vascular bundle and the rachis of point inoculated wheat spikelets above and below the PI have been suggested as the cause of the slower acropetal movement of the pathogen in the spike (9).

Rachis colonization was closely correlated with the severity of FHB symptoms and, to a lesser extent, with the relative TKW. Furthermore, colonization was highly correlated with the amount of DON present in wheat grains, but not with DON production *in vitro*. These results agree with those reported by Gilbert et al. (21), who found no correlation between severity of FHB, fungal biomass and *in vitro* DON production.

In more than 90% of the total evaluated spikes, the presence of the pathogen could be verified in the rachis portions that corresponded to the inoculated spikelets of the field grown spikes. Such percentage exceeded the 70% incidence observed when visible symptoms of the disease were analyzed in the field (36). According to these results, in a high proportion of inoculated spikes, the simple presence of *F. graminearum* infecting the rachis was not sufficient for the development of FHB symptoms. In this regard, Brown *et al.* (9) found evidence of a macroscopically asymptomatic stage in wheat spikes where FHB symptoms remained confined to point inoculated spikelets, though at the cellular level internal infection by *F. graminearum* was clearly visible.

The colonization of the rachis of asymptomatic spikes by the pathogen could explain the fact that for isolates that induced the expression of FHB symptoms in extremely low severity values DON levels detected in the harvested grain were often higher than those tolerated by commercialization standards. These results imply that even isolates with a limited capacity of induction of visible symptoms of FHB and reduction of TKW may be able to colonize the rachis of wheat spikes producing detectable amounts of DON.

The search for wheat genotypes tolerant or resistant to FHB, which could aid in lowering the impact of mycotoxin contamination of food and feed, constitutes a fundamental objective for breeders. The results obtained in our work allow hypothesizing that limiting the exposition of wheat genotypes to DON, as well as increasing the tolerance of the plant to trichothecenes could reduce the development of *F. graminearum* and, as a consequence, diminish the severity of FHB attacks. Recently, we reported that the presence of high levels of variability among the populations of *F. graminearum* prompted the need to include mixtures of isolates when screening for disease resistant genotypes (36). According to the results reported in the present work, the fact that even isolates with limited aggressiveness present an important risk of DON contamination emphasizes the need to use combinations of isolates that not only reflect the variable levels of aggressiveness, but also the variability in toxigenic potential present in the populations of the pathogen.

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Table 1 – Primers used for determination of the chemotype of 112 isolates of *Fusarium graminearum* from the Buenos Aires Province in Argentina by multiplex polymerase chain reaction (PCR).

Primer	**Sequence (5' to 3')**	**Chemotype**	**Target Gene**	**Amplified product (bp)**
3NA	GTG CAC AGA ATA TAC GAG C	NIV	*TRI3*	840
3D15A	ACT GAC CCA AGC TGC CAT C	15-ADON		610
3D3A	CGC ATT GGC TAA CAC ATG	3-ADON		243
3CON	TGG CAA AGA CTG GTT CAC			
12NF	TCT CCT CGT TGT ATC TGG	NIV	*TRI12*	840
12-15F	TAC AGC GGT CGC AAC TTC	15-ADON		670
12-3F	CTT TGG CAA GCC CGT GCA	3-ADON		410
12CON	CAT GAG CAT GGT GAT GTC			

Table 2 – Severity of Fusarium Head Blight (FHB) induced on field grown spikes of wheat cv. Klein Chajá, mycotoxin production and expansion in the rachis of 14 isolates of *Fusarium graminearum* from Buenos Aires province, Argentina.

Isolate	**Severity 21 dpi (%)**	**TKW (% of control)**	**DON *in vivo* (ppm)**	**DON *in vitro* (ppm)**	**Spike infection (%)**
SP1	50^{a}	65^{c}	31.3^{a}	227.8^{abc}	86^{a}
LF2	35^{b}	70^{abc}	16.2^{bc}	221.9^{abc}	69^{abcd}
BA15	28^{b}	79^{ab}	19.1^{ab}	316.9^{abc}	83^{ab}
BA14	27^{b}	82^{ab}	21.2^{ab}	238.2^{abc}	86^{a}
LH11	26^{b}	75^{abc}	21.4^{ab}	366.7^{a}	78^{abc}
30A3	25^{bc}	91^{ab}	14.3^{bc}	328.3^{abc}	69^{abcd}
LF1	25^{bc}	86^{ab}	16.2^{bc}	316.9^{abc}	77^{abc}
MR46	23^{bc}	80^{ab}	14.8^{bc}	373.0^{a}	81^{abc}
LH7	22^{bcd}	84^{ab}	14.7^{bc}	311.0^{abc}	80^{abc}
GA1	14^{bcd}	90^{ab}	13.0^{bc}	140.6^{bc}	60^{bcd}
MR40	14^{bcd}	87^{ab}	11.9^{bc}	357.9^{ab}	72^{abcd}
MR25	11^{bcd}	92^{ab}	12.7^{bc}	126.5^{c}	71^{abcd}
MR4	10^{cd}	91^{ab}	9.4^{bc}	244.7^{abc}	57^{cd}
MR18	6^{d}	94^{a}	4.5^{c}	177.2^{abc}	48^{d}
CONTROL	0^{d}	100^{a}	0.1^{c}	0^{c}	3^{e}

Different letters correspond to significative differences between treatments ($\alpha = 0.05$).

Figure 1 – (A) Plating on potato dextrose agar (PDA) culture medium of the upper rachis portion fragments of a wheat spike inoculated with an isolate of *Fusarium graminearum*, and (B) development of colonies of *F. graminearum* from the plated rachis fragments of the lower portion of another spike.

Figure 2 – Percentage of field grown wheat spikes point-inoculated with each of 14 isolates of *Fusarium graminearum* showing symptoms of Fusarium Head Blight, symptoms of premature bleaching, and colonization of rachis fragments by the fungus above and below the point of inoculation (PI). Positive numbers represent the spikelets located above the PI while negative numbers indicate those located below it (i.e. +0 and -0 are the inoculated spikelets and +1 and -1 the spikelets immediately above and below them, respectively).

Figure 3 – Percentage of field grown wheat spikes point inoculated with 14 isolates of *Fusarium graminearum* with rachis portions above and below the point of inoculation (PI) showing colonization by the fungus. Positive numbers represent the spikelets located above the PI while negative numbers indicate those located below it (i.e. +0 and -0 are the inoculated spikelets and +1 and -1 the spikelets immediately above and below them, respectively). Different letters indicate the existence of significative differences between treatments ($\alpha = 0.05$).

Figure 4 – Regression analysis between the severity of symptoms of Fusarium Head Blight induced by 14 isolates of *Fusarium graminearum* from Argentina point-inoculated on field-grown wheat spikes and deoxynivalenol (DON) concentration in the resulting wheat kernels.

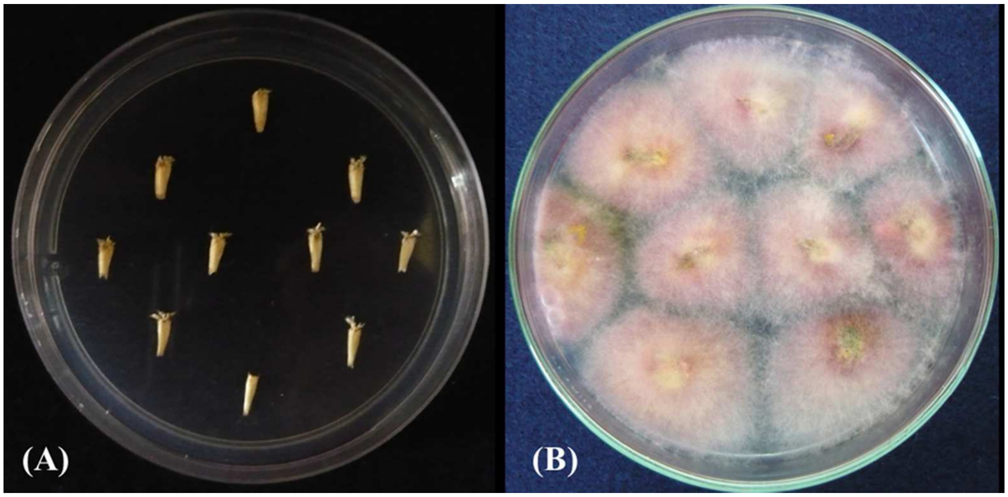

Figure 1 - (A) Plating on potato dextrose agar (PDA) culture medium of the upper rachis portion fragments of a wheat spike inoculated with an isolate of *Fusarium graminearum*, and (B) development of colonies of *F. graminearum* from the plated rachis fragments of the lower portion of another spike.
91x44mm (300 x 300 DPI)

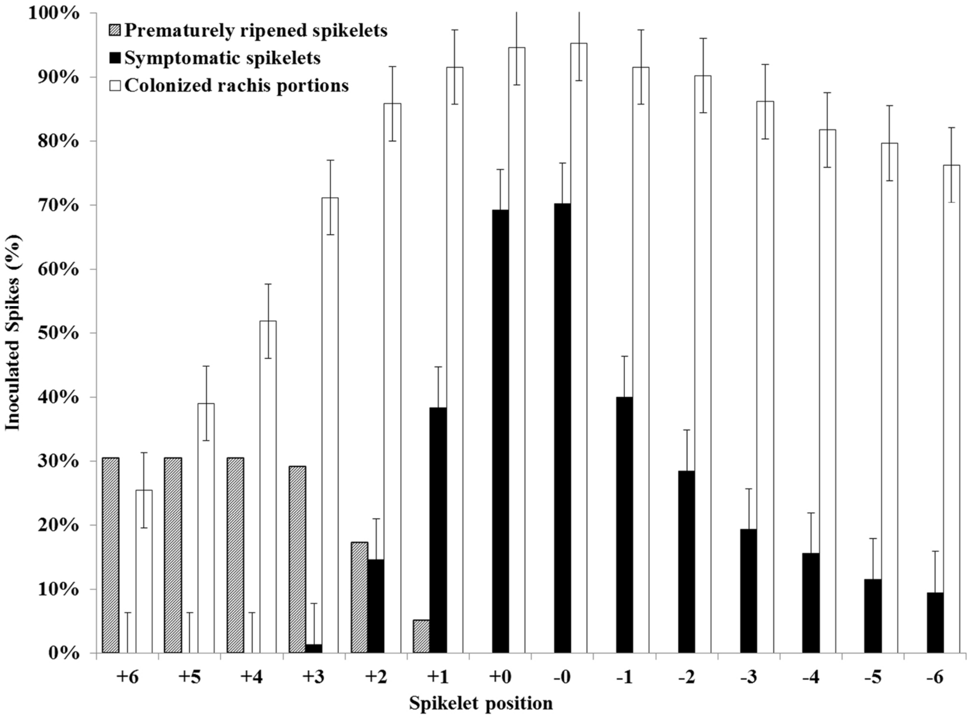

Figure 2 - Percentage of field grown wheat spikes point-inoculated with each of 14 isolates of *Fusarium graminearum* showing symptoms of Fusarium Head Blight, symptoms of premature bleaching, and colonization of rachis fragments by the fungus above and below the point of inoculation (PI). Positive numbers represent the spikelets located above the PI while negative numbers indicate those located below it (i.e. +0 and -0 are the inoculated spikelets and +1 and -1 the spikelets immediately above and below them, respectively).
174x126mm (300 x 300 DPI)

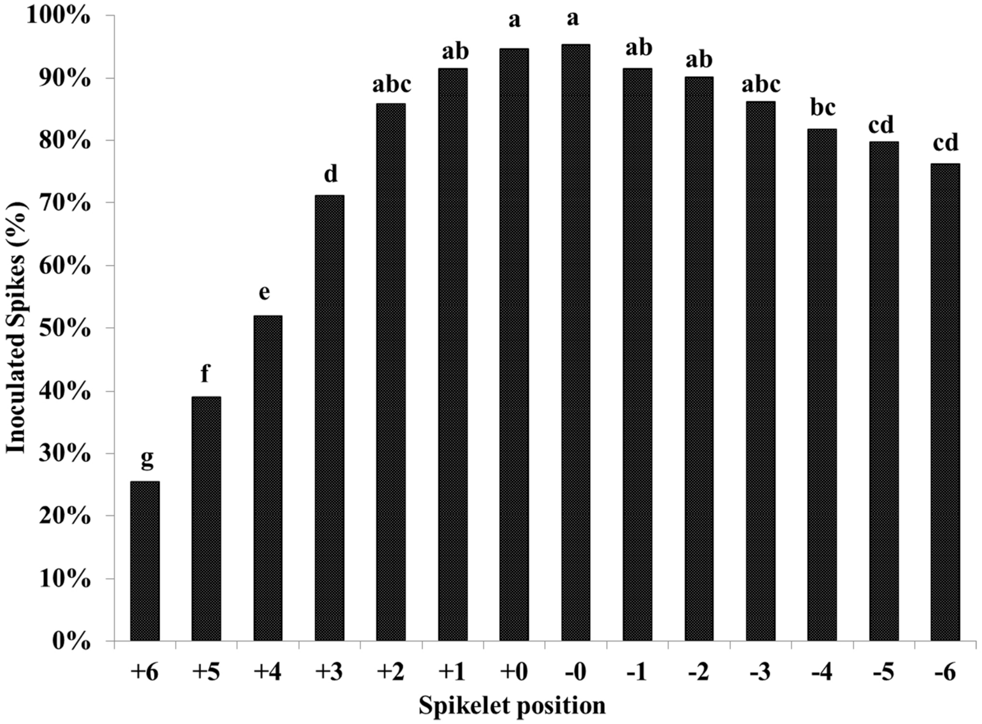

Figure 3 - Percentage of field grown wheat spikes point inoculated with 14 isolates of *Fusarium graminearum* with rachis portions above and below the point of inoculation (PI) showing colonization by the fungus. Positive numbers represent the spikelets located above the PI while negative numbers indicate those located below it (i.e. +0 and -0 are the inoculated spikelets and +1 and -1 the spikelets immediately above and below them, respectively). Different letters indicate the existence of significative differences between treatments (α = 0.05).
175x128mm (300 x 300 DPI)

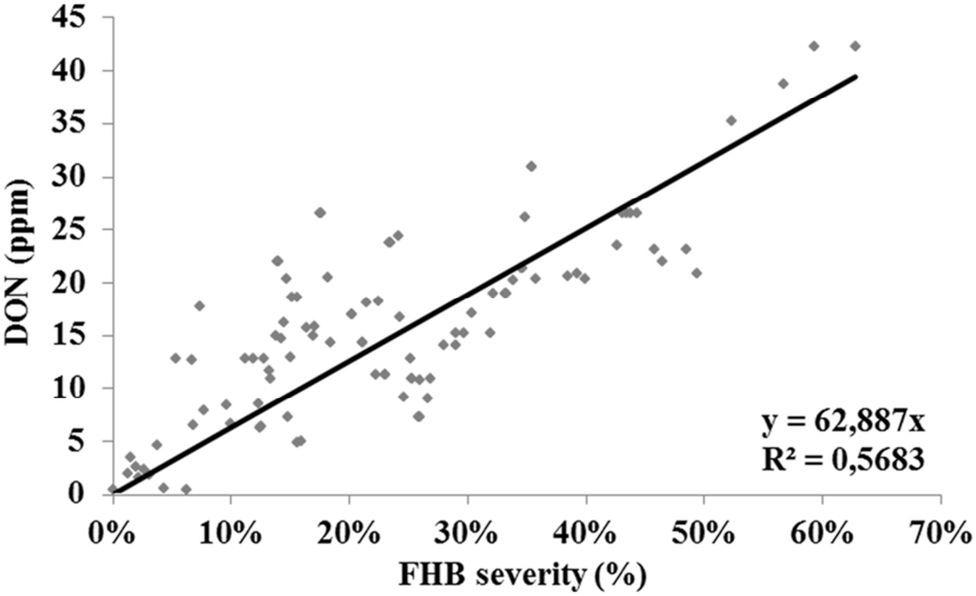

Figure 4 - Regression analysis between the severity of symptoms of Fusarium Head Blight induced by 14 isolates of *Fusarium graminearum* from Argentina point-inoculated on field-grown wheat spikes and deoxynivalenol (DON) concentration in the resulting wheat kernels.
76x45mm (300 x 300 DPI)