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Trichothecene genotypes and production profiles of *Fusarium graminearum* isolates obtained from barley cultivated in Argentina

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ABSTRACT

Fusarium graminearum is one of the most important pathogens isolated from small cereal grains with Fusarium Head Blight symptoms. The presence of this fungus is often linked to the occurrence of several mycotoxins in barley and wheat. The aim of our study was to characterize trichothecene genotypes and production profiles of *F. graminearum sensu stricto* isolates obtained from barley grains in Argentina. A total of 110 *F. graminearum s.s.* isolates were analyzed by PCR assays to predict deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON) and nivalenol (NIV) production, and all isolates were found to belong to the same molecular 15-ADON genotype. Trichothecene production in autoclaved rice was analyzed by using gas chromatography (GC) and confirmed by GC–MS. Of the 110 isolates, 95% were able to produce DON, 71% produced 15-ADON, 63% 3-ADON and 52% NIV. With the exception of a single isolate, all isolates that produced NIV, also produced DON. However, the NIV production was very low, ranging from 0.13 to 0.30 μg/g. Six different production profiles of DON and its acetyl-derivatives were detected, the predominant being simultaneous production of DON, 3-ADON and 15-ADON, followed by DON production, and DON and 15-ADON co-production. This work is the first attempt to characterize the trichothecene genotypes and production profiles of *F. graminearum s.s.* isolates from Argentinean barley.

1. Introduction

Barley (*Hordeum vulgare* L.) is the second main winter crop in Argentina, where its cropping area has increased to 1.5 million ha in the last growing season (2012–2013), with a production of ±5 million tons. The principal use of this crop is for malt production, although substandard grains are used for animal feed. *Fusarium graminearum* (syn. *Gibberella zeae*) is the principal causal agent of *Fusarium* Head Blight (FHB) on small cereal grains worldwide. Even though the main problem of the infection is yield loss, a cause of great concern is the ability of *F. graminearum* to contaminate the barley kernels with type B trichothecenes such as nivalenol (NIV) and its acetylated derivatives, and deoxynivalenol (DON), 15-acetyldeoxynivalenol (15-ADON) or 3-acetyldeoxynivalenol (3-ADON) (Desjardins, 2006). In general, DON is associated with vomiting, feed refusal and also quality loss of malt and beer gushing (Schwarz et al., 1996; Oliveira et al., 2012), whereas NIV is more toxic to humans and animals than DON (Minervini et al., 2004).

In recent years, several PCR assays have been developed to predict *F. graminearum*/*F. culmorum* chemotypes based on the sequences of the trichothecene biosynthesis pathway genes. For example, primers to differentiate DON from NIV producers based on sequence alleles at *Tri7* and *Tri13* were developed by Lee et al. (2001, 2002) and Chandler et al. (2003), primers based on *Tri5* and *Tri6* gene sequences were developed to differentiate high or low DON producers (Bakan et al., 2002); *Tri3*, *Tri5*, and *Tri7* gene sequences were used to design primers used to differentiate 15-ADON, 3-ADON and NIV producers, respectively (Quarta et al., 2006). Three different chemotypes have been described in *F. graminearum* corresponding to different trichothecene profiles: NIV chemotype, when NIV is produced; 15-ADON chemotype, when DON and 15-ADON are produced; 3-ADON chemotype, when DON and 3-ADON are produced (Desjardins, 2006). On the other hand, such classification should be correctly referred to genotypes since it is based on DNA sequences (Desjardins, 2008). Trichothecene chemotype definition should only be used when the chemical phenotype is expressed and detected by chemical analyses, because the detection of a given trichothecene genotype does not always predict the presence

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of the corresponding metabolites (Desjardins, 2008). Genotyping results are usually well in accordance with chemotypes (e.g. Ward et al., 2002; Quarta et al., 2006; Yli-Mattila et al., 2009; Reynoso et al., 2011).

The toxigenic potential of *F. graminearum sensu stricto* strains varies around the world. In France, Germany, Italy, Luxembourg, and Turkey, 15-ADON producers were higher in frequency than those of NIV or 3-ADON (Pasquali et al., 2010; Mugrabi de Kuppler et al., 2011; Yörük and Albayrak, 2012; Boutigny et al., 2014; Somma et al., 2014). However, studies made in different regions of Russia and adjacent countries showed that 3-ADON producing isolates of *F. graminearum* are dominant in northern Europe. The 3-ADON chemotype was found to be prevalent in Scandinavia, Finland and north-western Russia, whereas the 15-ADON chemotype was more common in southern Europe and China. Both 3-ADON and 15-ADON chemotypes are common in the Russian Far East (Jestoi et al., 2008; Yli-Mattila et al., 2009; Yli-Mattila, 2010). *F. graminearum* isolates with the 15-ADON genotype were found in previous studies as largely responsible for FHB in North America. More recent studies indicated localized heterogeneity among *F. graminearum* populations (Ward et al., 2008). Gale et al. (2011) observed that NIV, 15-ADON and 3-ADON genotype populations obtained from wheat varied according to the geographic origin in the USA. In South America (Uruguay and Brazil) *F. graminearum* isolates of the 15-ADON genotype were reported to predominate in wheat (Scoz et al., 2009; Pan et al., 2013).

In Argentina *F. graminearum s.s.* studies regarding the genotype and/or the production of trichothecenes NIV, DON, 15-ADON, 3-ADON have focused on wheat. Reynoso et al. (2011) using a multiplex PCR assay found that the 92% of the *F. graminearum* isolates evaluated had the 15-ADON genotype. Fernandez Pinto et al. (2008), Alvarez et al. (2009) and Reynoso et al. (2011) revealed that the most common profile of trichothecene production was DON + 15-ADON, although 3-ADON and NIV producers were also detected.

Genotype and chemical profile surveys of *F. graminearum s.s.* isolated from barley have not been previously carried out in Argentina. There have been few studies analyzing *F. graminearum* isolates obtained from barley around the world. Yang et al. (2008) found that most of the isolates from Chinese barley belonged to the DON molecular chemotype (genotype). Astolfi et al. (2011) and Boutigny et al. (2014) reported that 15-ADON genotype was predominant in barley from Southern Brazil and France, respectively.

The aim of our study was to characterize trichothecene genotypes and chemical profiles of *F. graminearum s.s.* isolates obtained from barley grains from different fields in the main production region of Argentina.

2. Materials and methods

2.1. Fungal isolation

F. graminearum isolates were obtained from different barley grain samples collected from commercial fields of Argentina during the 2010, 2011 and 2012 growing seasons (Table 1). Grain samples (200 g) were reduced to 400 grains with a grain divider, surface-disinfected, placed onto potato dextrose agar (PDA) with 0.25 g chloramphenicol/L and incubated for 4–7 days at 25 ± 2 ºC under a 12 h light/dark cycle. Single spore *F. graminearum* isolates were morphologically identified on PDA and on Spezieller Nährstoffarmer Agar (SNA) according to Leslie and Summerell (2006).

2.2. DNA isolation

Genomic DNA from a total of 110 monosporic *F. graminearum* isolates (Table 1) was extracted using the cetyltrimethylamonium bromide (CTAB) method according to Stenglein and Balatti (2006). The quality of fungal DNA was examined by electrophoresis in 0.8% (w/v) agarose gels containing GelRed™ (Biotium, Hayward, USA) at 80 V in 1× Trisborate-EDTA buffer for 3 h at room temperature. The DNA was visualized under UV light. The DNA concentration was estimated with a fluorometer (Qubit™-Invitrogen, Buenos Aires, Argentina).

2.3. Polymerase chain reaction assays

2.3.1. Fusarium graminearum sensu stricto identification

To confirm morphological identifications a *F. graminearum*-specific PCR was performed for the 110 isolates using primers Fg16F and Fg16R according to Nicholson et al. (1998). These primers are not completely specific to *F. graminearum s.s.*, but they give products of different size. *F. graminearum* gives a product of about 400 bp, while *F. asiaticum* (NRRL 13818, used as control) gives a PCR product of about 550 bp and *F. meridionale* (NRRL 28436, used as control) gives a product of about 500 bp.

PCR assays were carried out using 10–20 ng of DNA in a total volume of 25 μL containing 10× reaction buffer, 0.5 μM of each primer, 200 μM of each dNTP (Genbiotech S.R.L.), 2.5 mM $MgCl_2$, and 1.25 U of *Taq* DNA polymerase (Inbio-Highway, Tandil, Argentina). DNA amplifications were performed in a XP thermal cycler (Bioer Technology Co.).

2.3.2. Genotype determination

PCR-trichothecene genotypes were classified into NIV or DON with primers Tri13NIVF/Tri13R and Tri13DONF/Tri13R, respectively, according to Chandler et al. (2003). NIV, DON, 15-ADON, and 3-ADON were also determined by a multiplex-PCR for all isolates with primers Tri7F340/Tri7R965, 3551H/4056H, Tri3F971/Tri3R1679 and Tri3F1325/Tri3R1679, respectively (Quarta et al., 2006).

F. graminearum Cv1.2 (15-ADON genotype), *F. graminearum* Cv12-C (3-ADON), and *F. meridionale* Cv811.1 (NIV) were used as DNA positive controls for trichothecene genotypes (Astolfi et al., 2011). The DNA of the isolates was kindly provided by PhD. Del Ponte, Departamento de Agronomia, Universidade Estadual de Maringá, Maringá, Brazil.

PCR products were examined by electrophoresis in 1.5% (w/v) agarose gels containing GelRed™ (Biotium, Hayward, USA) at 80 V in 1× Trisborate-EDTA buffer for 2 h. Fragments were visualized under UV light. Each PCR reaction was performed at least twice, running positive controls, plus the DNA ladder (100 bp) and the negative control in one gel, simultaneously.

2.4. Trichothecene analysis

Toxin analysis was performed according to Alvarez et al. (2009). Briefly, 250-mL Erlenmeyer flasks containing 25 g of sterilized rice and 15 mL of sterile distilled water were inoculated with each monosporic isolate. To determine the absence of trichothecenes in the rice, negative controls (triplicate) were prepared in the same way without inoculation. The inoculated flasks as well as the controls were incubated at 25 °C for 14 days and at 10 °C for 14 days in the dark.

Trichothecenes were extracted for 1 h at 300 rpm with 125 mL of acetonitrile:acetylacetate:water (50:41:9). The clean-up was performed with a column packed with charcoal:alumina:celite (0.7:0.5:0.3) and dried in Rotavap®. Gas chromatography, with ^{63}Ni electron capture detection Shimadzu Model GC17 equipped with split/splitless injector and fitted with RX-5MS capillary column (25 m × 0.2 mm id), were used to detect and quantify trichothecenes. The detection limits were 0.02 μg g^{-1} for DON and its acetyl derivatives, and 0.05 μg/g for NIV. Standards of DON, 15-ADON, 3-ADON and NIV were purchased from SIGMA Chemical Company (St Louis, MO, USA).

The presence of compounds was confirmed by Gas Chromatography–Mass spectrometer system (GC–MS QP 5050A, Shimadzu®) with Electron Impact (EI) mode (70 eV) as described by Alvarez et al. (2009).

Table 1
Origin and mycotoxin production of *Fusarium graminearum* isolates.

Isolate[a]	Year/locality	Barley variety	NIV μg/g	DON μg/g	3-ADON μg/g	15-ADON μg/g
1-1	2010/S.Spiritu	Scarlett	n.d.	4.22	0.37	2.11
3-4	2010/Tapalqué	Scarlett	0.14	88.85	3.80	n.d.
3-6	2010/Tapalqué	Scarlett	n.d.	17.90	7.15	1.30
6-2	2010/Azul	Shakira	n.d.	0.23	n.d.	n.d.
6-3	2010/Azul	Shakira	n.d.	66.48	1.25	8.04
11-1	2010/Azul	Scarlett	0.14	5.25	0.30	0.20
11-2	2010/Azul	Scarlett	n.d.	45.60	1.00	1.10
13-1	2010/Azul	Scarlett	0.14	14.30	1.00	n.d.
13-2	2010/Azul	Scarlett	0.13	9.65	1.00	0.60
13-3	2010/Azul	Scarlett	0.14	363.50	8.60	21.50
13-4	2010/Azul	Scarlett	0.27	701.30	3.20	n.d.
13-5	2010/Azul	Scarlett	0.14	12.45	0.30	3.05
13-6	2010/Azul	Scarlett	n.d.	43.02	3.90	1.94
17-1	2010/Olavarría	Scarlett	n.d.	19.27	5.52	3.11
23-4	2011/T.Lauquen	Scarlett	n.d.	58.10	4.73	0.76
27-3	2011/Azul	Shakira	0.13	8.90	n.d.	n.d.
27-1	2011/Azul	Shakira	n.d.	2.25	4.55	0.60
27-2	2011/Azul	Shakira	n.d.	4.97	n.d.	0.36
27-5	2011/Azul	Shakira	0.14	13.00	0.75	0.35
27-9	2011/Azul	Shakira	0.14	18.55	0.75	1.25
27-10	2011/Azul	Shakira	0.30	34.20	2.42	0.58
27-14	2011/Azul	Shakira	0.14	21.65	0.28	0.10
27-16	2011/Azul	Shakira	0.16	25.25	4.40	0.55
27-17	2011/Azul	Shakira	0.14	5.37	3.75	5.40
27-18	2011/Azul	Shakira	0.14	4.97	4.80	0.75
27-20	2011/Azul	Shakira	0.16	49.14	n.d.	0.80
29-5	2011/Azul	Scarlett	n.d.	83.23	2.33	0.79
29-7	2011/Azul	Scarlett	0.15	18.21	0.15	7.90
29-9	2011/Azul	Scarlett	n.d.	6.25	n.d.	n.d.
29-11	2011/Azul	Scarlett	n.d.	22.20	0.20	n.d.
29-12	2011/Azul	Scarlett	0.14	2.72	n.d.	5.35
29-14	2011/Azul	Scarlett	0.27	21.80	0.70	n.d.
29-15	2011/Azul	Scarlett	0.15	205.60	1.15	5.50
32-4	2011/Chillar	Scarlett	0.14	29.80	0.10	0.30
33-2	2011/Tandil	Scarlett	0.14	27.80	n.d.	3.65
33-4	2011/Tandil	Scarlett	0.13	1.20	n.d.	10.85
33-9	2011/Tandil	Scarlett	n.d.	6.84	1.66	0.14
33-10	2011/Tandil	Scarlett	n.d.	11.42	1.18	0.20
33-11	2011/Tandil	Scarlett	0.14	19.80	0.85	0.80
33-14	2011/Tandil	Scarlett	n.d.	1.18	0.63	n.d.
33-16	2011/Tandil	Scarlett	n.d.	n.d.	0.12	n.d.
33-17	2011/Tandil	Scarlett	0.14	5.86	1.15	2.25
33-20	2011/Tandil	Scarlett	0.28	3.90	n.d.	0.09
33-22	2011/Tandil	Scarlett	n.d.	4.08	n.d.	2.04
33-24	2011/Tandil	Scarlett	0.27	5.50	n.d.	n.d.
33-27	2011/Tandil	Scarlett	0.27	1.95	n.d.	n.d.
33-28	2011/Tandil	Scarlett	n.d.	16.92	n.d.	n.d.
33-29	2011/Tandil	Scarlett	0.14	4.35	2.45	0.10
33-30	2011/Tandil	Scarlett	n.d.	75.40	n.d.	1.18
33-31	2011/Tandil	Scarlett	0.27	4.02	n.d.	n.d.
33-33	2011/Tandil	Scarlett	n.d.	n.d.	n.d.	0.18
35-4	2011/La Madrid	Scarlett	n.d.	n.d.	n.d.	0.33
62-1	2011/Azul	Scarlett	0.14	2.45	5.50	0.05
62-3	2011/Azul	Scarlett	n.d.	2.70	n.d.	0.11
62-9	2011/Azul	Scarlett	0.16	18.91	0.22	15.05
62-10	2011/Azul	Scarlett	0.14	94.75	0.22	24.54
68-1	2011/Tandil	Scarlett	n.d.	55.80	0.32	0.38
68-3	2011/Tandil	Scarlett	n.d.	9.45	0.55	n.d.
68-5	2011/Tandil	Scarlett	n.d.	60.73	0.99	0.23
68-6	2011/Tandil	Scarlett	0.14	87.72	n.d.	0.15
68-9	2011/Tandil	Scarlett	0.14	10.30	n.d.	0.80
68-10	2011/Tandil	Scarlett	0.29	3.39	11.20	4.90
68-4	2011/Tandil	Scarlett	0.27	6.45	n.d.	n.d.
68-8	2011/Tandil	Scarlett	0.15	202.51	n.d.	5.05
82-1	2011/Azul	Scarlett	0.14	7.17	3.55	7.55
83-1	2011/Azul	Scarlett	n.d.	n.d.	0.52	n.d.
85-1	2011/Azul	Scarlett	0.27	n.d.	n.d.	n.d.
87-2	2011/Azul	Scarlett	0.27	1.40	n.d.	n.d.
87-3	2011/Azul	Scarlett	0.14	6.39	2.00	2.10
87-4	2011/Azul	Scarlett	0.15	64.80	2.50	2.75
87-5	2011/Azul	Scarlett	n.d.	7.27	0.79	0.13
87-1	2011/Azul	Scarlett	0.30	7.38	n.d.	n.d.
87-7	2011/Azul	Scarlett	n.d.	5.08	n.d.	n.d.
88-1	2011/Azul	Scarlett	n.d.	421.93	15.90	2.10

(continued on next page)

Table 1 (*continued*)

Isolate[a]	Year/locality	Barley variety	NIV μg/g	DON μg/g	3-ADON μg/g	15-ADON μg/g
88-2	2011/Azul	Scarlett	0.14	431.50	n.d.	4.70
88-3	2011/Azul	Scarlett	n.d.	158.90	n.d.	n.d.
90-1	2011/Azul	Scarlett	n.d.	136.90	1.29	4.44
90-2	2011/Azul	Scarlett	n.d.	149.70	1.33	0.13
92-1	2011/Olavarría	Scarlett	n.d.	28.65	n.d.	n.d.
92-2	2011/Olavarría	Scarlett	n.d.	914.00	3.28	2.55
92-8	2011/Olavarría	Scarlett	n.d.	24.22	0.74	0.67
92-10	2011/Olavarría	Scarlett	0.14	31.04	1.40	4.20
92-11	2011/Olavarría	Scarlett	0.28	22.10	1.27	1.80
92-7	2011/Olavarría	Scarlett	0.14	22.40	7.20	6.95
92-6	2011/Olavarría	Scarlett	n.d.	30.49	n.d.	0.10
114-1	2012/Lobería	NN	n.d.	104.05	2.50	2.10
114-2	2012/Lobería	NN	n.d.	68.32	n.d.	0.20
116-1	2012/Necochea	NN	n.d.	8.11	4.61	0.62
119-6	2012/San Cayetano	NN	n.d.	17.43	5.07	0.61
119-8	2012/San Cayetano	NN	n.d.	228.46	0.67	0.22
119-9	2012/San Cayetano	NN	n.d.	20.40	0.63	0.09
119-12	2012/San Cayetano	NN	n.d.	0.41	n.d.	n.d.
119-14	2012/San Cayetano	NN	0.27	0.86	n.d.	n.d.
119-17	2012/San Cayetano	NN	n.d.	0.54	4.45	0.65
119-18	2012/San Cayetano	NN	n.d.	50.30	3.36	1.21
119-2	2012/San Cayetano	NN	n.d.	10.49	n.d.	n.d.
119-30	2012/San Cayetano	NN	n.d.	41.05	4.33	0.88
119-32	2012/San Cayetano	NN	n.d.	2.63	n.d.	n.d.
119-33	2012/San Cayetano	NN	0.27	2.16	n.d.	n.d.
119-39	2012/San Cayetano	NN	0.13	7.75	n.d.	0.50
119-36	2012/San Cayetano	NN	n.d.	6.12	1.25	0.12
120-1	2012/Tres Arroyos	NN	0.14	9.37	20.00	17.25
120-2	2012/Tres Arroyos	NN	0.27	0.45	n.d.	n.d.
129-1	2012/Azul	Scarlett	n.d.	642.86	5.72	1.33
129-2	2012/Azul	Scarlett	0.14	20.46	4.85	15.40
129-3	2012/Azul	Scarlett	0.27	1.74	n.d.	n.d.
129-4	2012/Azul	Scarlett	0.27	4.75	n.d.	n.d.
129-5	2012/Azul	Scarlett	n.d.	34.75	3.61	0.44
129-6	2012/Azul	Scarlett	n.d.	3.44	3.56	1.45
130-1	2012/Azul	B1215	0.27	7.40	1.60	n.d.

NN = data not available.
n.d. = not detected.
[a] The first number identify different samples and the number next to the dash identify different isolates of a sample.

2.5. Statistical analysis

Exploratory statistical analysis, histograms and Shapiro Wilk test were done with Statistica software v 8.0 (StatSoft, Inc., 1984–2007, Tulsa, OK, USA).

3. Results

3.1. Trichothecene genotype analyses by PCR

All *F. graminearum* isolates produced a fragment of ≈400 bp characteristic of the species, confirming morphological identifications.

All positive controls amplified the specific fragment according to the primers used in the PCR assays. The 110 *F. graminearum* isolates used for this study amplified a fragment of 282 bp with primers Tri13F/Tri13DONR and no amplifications were observed for primers Tri13NIVF/Tri13R, and were classified as representatives of the DON genotype. All isolates amplified fragments of 525 bp and 708 bp in the multiplex-PCR reactions and were classified as 15-ADON genotype.

3.2. Trichothecene chemical analyses

The chemical analysis of the *F. graminearum* isolates obtained from barley grains demonstrated their capacity to produce high amounts of the evaluated toxins, especially DON (Table 1). DON was produced by 95% of the isolates, 71% produced 15-ADON, 63% 3-ADON, and 52% NIV. NIV was found simultaneously with DON, but in much lower quantities, except for isolate 85-1 that produced only NIV, also in low amount (Table 1). Except for a low number of isolates (10 of 96), DON was always produced in larger amounts than the other analyzed trichothecenes. Table 2 shows the average amount and range of production for each toxin.

3.3. Statistical analyses

According to the chemical analysis of the isolates, the results of the present work suggest that DON is the main trichothecene to be found in Argentinean barley grains, and it is expected to be present in high concentrations. However, it is also probable to find both 3-ADON and 15-ADON in lower quantities. The production of both DON acetyl-derivatives by all *F. graminearum* isolates followed a similar non-Gaussian distribution (Fig. 1) with close means and standard deviations (3-ADON, Shapiro Wilk test $p < 0.0001$, $n = 110$, mean = 1.85, SD = 3.10; 15-ADON, Shapiro Wilk test $p < 0.0001$, $n = 110$, mean = 2.10, SD = 4.27). The maximum levels of production were also similar for both 3-ADON and 15-ADON (20.0 and 24.5 μg/g respectively), which suggest that the isolates able of producing any of the acetyl-derivatives are alike in their biosynthesis capacity.

Table 2
Maximum, minimum and mean concentrations of trichothecenes produced by *Fusarium graminearum* isolated from barley.

	NIV (μg/g)	DON (μg/g)	3-ADON (μg/g)	15-ADON (μg/g)
Maximum	0.30	914.00	20.00	24.54
Minimum	0.13	0.23	0.10	0.05
Average	0.19	62.28	2.91	2.96

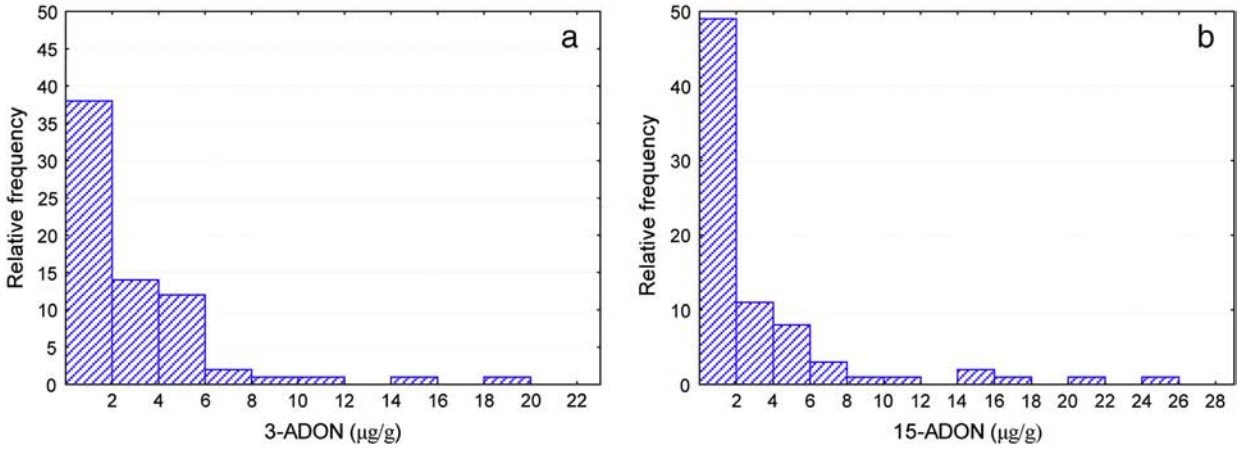

Fig. 1. Relative frequency of a) 3-acetyldeoxynivalenol and b) 15-acetyldeoxynivelenol concentration produced by *Fusarium graminearum* isolated from barley.

3.4. Profiles of production of DON and acetyl-derivatives by F. graminearum sensu stricto isolated from barley

According to DON and their acetyl-derivatives biosynthesis, six different profiles of production were detected among the *F. graminearum s.s.* isolates from barley (Table 3). The predominant one was characterized by the production of DON + 3-ADON + 15-ADON. In much lower quantities, the second and third in proportion were the DON and DON + 15-ADON. Simultaneous production of DON + 3-ADON was also observed, although in lower frequency. Only two out of 110 isolates were producers of 15-ADON or 3-ADON alone.

Although different barley varieties (Scarlett, Shakira and B1215), years and/or geographical localities were sampled, the *F. graminearum* isolates analyzed did not present a clear pattern of mycotoxin production related to any of these variables. Moreover, in a single sample we found *F. graminearum* isolates with a different pattern of trichothecene production (e.g., samples 33, 92) (Table 1).

4. Discussion

All 110 isolates of *F. graminearum sensu stricto* isolated from barley analyzed in the present study belonged to the 15-ADON genotype. Different studies around the world have been published in relation to the capacity (genotype level) of isolates of *F. graminearum s.s* from small cereals to produce trichothecenes. Little is known about *Fusarium* populations isolated from barley grains. In China, *F. asiaticum* was reported as the predominant species causing FHB on barley, and the NIV genotype was the most abundant. However, the *F. graminearum s.s.* isolates isolated from barley, present in lower proportion, belonged to the DON molecular chemotype (Yang et al., 2008). In recent surveys of *Fusarium* trichothecene genotype from barley grains in France and Brazil, the 15-ADON genotype was the most frequent among *F. graminearum s.s.* isolates, but 3-ADON genotype was also present (Astolfi et al., 2011; Boutigny et al., 2014). Our results agree with the prevalence of 15-ADON genotype in barley grains in South America.

Table 3
Profiles of deoxynivalenol and acetyl-derivative production by *Fusarium graminearum* isolated from barley.

Trichothecene profile	DON	3-ADON	15-ADON	No. of isolates	Percentage of isolates
I	+	+	+	60	54.5
II	+	−	−	21	19.1
III	+	−	+	16	14.5
IV	+	+	−	8	7.3
V	−	+	−	2	1.8
VI	−	−	+	2	1.8

There is no information on *F. graminearum* genotypes/chemical production profiles of isolates from barley in Argentina. With respect to *Fusarium* populations from different small cereal crops in Argentina, studies in the wheat cropping area reported that *F. graminearum* isolates were predominantly 15-ADON producers (Fernandez Pinto et al., 2008; Alvarez et al., 2009; Reynoso et al., 2011). The 15-ADON chemotype was also predominant in *F. graminearum* isolates from soybean (Barros et al., 2012).

Chemical analysis of the *F. graminearum s.s.* isolates demonstrated differences with our PCR-genotype results. All isolates showed a positive PCR reaction for DON and the chemical results showed that the 95% produced DON in different concentrations. This discrepancy between genotype and chemical analyses has also been reported in the literature (Desjardins, 2008; Somma et al., 2014) and could be explained by the fact that PCR assays determine the potential to produce a mycotoxin at the genome level, but the in vitro production of a toxin could change with growth conditions.

A considerable number of isolates that amplified and produced DON were able to produce low quantities of NIV, but no PCR reaction for NIV was detected in our PCR assays. This result was also reported by Mugrabi de Kuppler et al. (2011), who found that DON genotype isolates tended to produce low amounts of NIV, and that DON was produced by isolates of the NIV genotype, although this characteristic could not be detected by the existing molecular assays. Alvarez et al. (2009) and Fernandez Pinto et al. (2008) also detected by chemical analysis that some of the isolates producing DON could produce low levels of NIV. Mugrabi de Kuppler et al. (2011) suggested that this situation could be attributed to the enzymatic conversion step of DON to NIV and vice versa or as a by-product of the biosynthesis.

In the present study, DON, 15-ADON and 3-ADON were produced simultaneously by 60 (54.5%) isolates. Moreover, 30 of these 60 isolates produced both acetyl-derivatives in similar amounts. Surprisingly, 22/110 isolates produced 3-ADON in a relationship of 4:1 or more than 15-ADON, and 10/110 produced 3-ADON and not 15-ADON, even though the 110 isolates were characterized molecularly as 15-ADON genotype. The discrepancy found between genotype and chemical data confirms that genotyping alone is not sufficient to predict the total profile of trichothecenes that isolates could synthesize, as was also found by Mugrabi de Kuppler et al. (2011).

The multiplex-PCR used for the differentiation of 3-ADON and 15-ADON genotypes used primers that amplified the *Tri*3 region. Recently, Alexander et al. (2011) postulated that *Tri*3 is not a determinant region to discriminate between 3-ADON and 15-ADON genotypes, and that *Tri8* was responsible for this differentiation. *Tri8* is required for

conversion of 3,15-diADON to either 3-ADON or 15ADON; in 3-ADON isolates, the *Tri8* enzyme is a trichothecene C-15 esterase (deacetylase), whereas in 15-ADON isolates is a C-3 esterase. According to their results, the differential activity of *Tri8* rather than lack of *Tri3* or *Tri8* activity in some isolates is determinant of 3-ADON or 15-ADON chemotypes. The authors suggest that low production of 3-ADON in an isolate genetically characterized as 15-ADON producer is most likely due to the activity of *Tri101*, a C-3 acetyltransferase acting on DON. As DON is not the preferred substrate of *Tri101*, the conversion to 3-ADON is slow. On the other hand, the production of low amounts of 15-ADON by 3-ADON genetically characterized isolates could be explained by *Tri3* acetylation of DON at C-15. DON is again a poor substrate for *Tri3*, and this would justify the low production. According to their results, *Tri8* is specific for deacetylation at C3 or C-15 in 15-ADON or 3-ADON isolates, respectively. It still remains to investigate if the *Tri8* activity is the basis for isolates producing both 3-ADON and 15-ADON in relatively similar amounts. If *Tri8* could have both activities, i.e. catalyze C-3 and C-15 deacetylation of 3,15-ADON, it seems possible that an isolate that can produce DON could also produce both acetyl derivatives if *Tri8* activity is interrupted or the enzyme does not completely catalyze both reactions.

The simultaneous production of 3-ADON and 15-ADON in comparable amounts by *F. graminearum s.s.* was also reported by Szécsi et al. (2005), Fernandez Pinto et al. (2008) and Alvarez et al. (2009). Previously, Sugiura et al. (1990) had reported the co-production of both DON acetyl-derivatives by *G. zeae* isolates isolated from barley and wheat from Japan. Interestingly, all these studies have in common that the incubation of the isolates for trichothecene production was made at two different temperatures. Sugiura et al. (1990) found differences in the DON acetyl-derivatives production pattern when the cultures were incubated at 25 °C for 21 days in comparison with incubating 2 weeks at 25 °C followed by two weeks at 4 °C. The first incubation conditions yielded DON and both acetyl-derivatives in similar amounts, while no 3-ADON was detected at the second set of conditions. The incubation pattern for the rest of the above mentioned studies was 2 weeks at 10 °C followed by 2 weeks at 25 °C, and all of them found a representative number of isolates that could accumulate similar quantities of both acetyl-derivatives. As environmental conditions have the potential to dramatically affect secondary metabolite biosynthesis, this could be a possible explanation for the results observed in these studies, as well as what was observed with the isolates from Argentinean barley in the present work, since the incubation conditions are the same. Sugiura et al. (1990) proposed an enzymatic activity that catalyzes the conversion of 3-ADON to 15-ADON regulated by temperature. It is also possible that, if *Tri8* could have the dual activity of catalyzing C-3 and C-15 deacetylation of 3,15-ADON, to yield 15-ADON and 3-ADON respectively, it was regulated by temperature or other environmental conditions. Accordingly, the discrepancy between genotype and chemical analyses in the present work could be explained by the dependence on growing conditions. Therefore, both types of analysis are necessary to reflect the potential contamination with trichothecenes in barley grains. Further studies on the relationship between environmental factors, substrate, culture conditions, etc. and DON acetyl-derivatives formation are needed. However, the development of new primer sets could be necessary to differentiate these acetyl-derivatives genotypes based on the *Tri8* region.

Different production profiles of *F. graminearum* isolates were found at the same location, and even in a single barley grain sample. This observation is important in relation with the type/s and amount of mycotoxins that could be found in the same or different samples. This work revealed for the first time the potential (genotype) and production profile of trichothecenes of *F. graminearum s.s.* isolates obtained from barley grains in Argentina. Knowledge of the mycotoxin potential and production of this important fungus on this economically relevant crop is necessary for risk assessments. Furthermore, continuous monitoring is required to understand and predict future changes in the toxigenic potential of *Fusarium* populations.

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