RESEARCH LETTER

# Development of a PCR assay to detect the potential production of nivalenol in *Fusarium poae*

María I. Dinolfo[1], Germán G. Barros[2,3] & Sebastián A. Stenglein[1,3,4]

[1]Laboratorio de Biología Funcional y Biotecnología (BIOLAB)-CEBB, Facultad de Agronomía de Azul, UNCPBA, Azul, Buenos Aires, Argentina; [2]Departamento de Microbiología e Inmunología, Universidad Nacional de Río Cuarto, Río Cuarto, Córdoba, Argentina; [3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina; and [4]Cátedra de Microbiología, Facultad de Agronomía de Azul, UNCPBA, Azul, Buenos Aires, Argentina

**Correspondence:** Sebastián A. Stenglein, Laboratorio de Biología Funcional y Biotecnología (BIOLAB)-CEBB, Facultad de Agronomía de Azul, UNCPBA, Av. República de Italia # 780. 7300 Azul, Buenos Aires, Argentina. Tel./fax: +54 2281 433291; e-mail: stenglein@faa.unicen.edu.ar

Received 13 February 2012; revised 19 April 2012; accepted 20 April 2012.
Final version published online 15 May 2012.

DOI: 10.1111/j.1574-6968.2012.02581.x

Editor: Jan Dijksterhuis

**Keywords**
*Fusarium* head blight; cereal grains; mycotoxins; rapid detection.

## Abstract

*Fusarium* species can produce mycotoxins, which can contaminate cereal-based food producing adverse effects for human and animal health. In recent years, the importance of *Fusarium poae* has increased within the *Fusarium* head blight complex. *Fusarium poae* is known to produce trichothecenes, especially nivalenol, a potent mycotoxin able to cause a variety of toxic effects. In this study, a specific primer pair was designed based on the *tri7* gene to detect potential nivalenol-producing *F. poae* isolates. A total of 125 *F. poae*, four *F. cerealis*, two *F. culmorum*, one *F. langsethiae*, one *F. sporotrichioides* and seven *F. graminearum*, plus *F. austroamericanum*, *F. meridionale*, *F. graminearum sensu stricto* and *F. cortaderiae* from the NRRL collection were analysed, and only *F. poae* isolates gave a positive result for the presence of a 296-bp partial *tri7* DNA fragment. Moreover, the primer set was tested from cereal seed samples where *F. poae* and other *Fusarium* species with a negative result for the specific reaction (*F. graminearum*, *F. oxysporum*, *F. chlamydosporum*, *F. sporotrichioides*, *F. equiseti* and *F. acuminatum*) were isolated, and the expected fragment was amplified. We developed a rapid and reliable PCR assay to detect potential nivalenol-producing *F. poae* isolates.

## Introduction

*Fusarium* head blight (FHB) is a disease of cereals caused by a complex of filamentous ascomycete fungi of genera *Fusarium* with a worldwide distribution (Stenglein, 2009). *Fusarium* species have a severe impact, reducing the yield and quality of seeds on diverse cereals such as wheat, barley, oat and corn (Kulik *et al.*, 2007). In addition, many species of the genus can produce mycotoxins, which are toxic metabolites that contaminate agricultural products along food production and can produce adverse effects for human and animal health (Moreno *et al.*, 2009).

*Fusarium* species are able to produce certain toxins such as fumonisin, enniatin, beauvericin, fusarin, moniliformin, fusaric acid, fusaproliferin and trichothecenes (Desjardins, 2006). Trichothecenes are tricyclic sesquiterpenes and some *Fusarium* species can produce the type A and/or the type B. Type A, such as T-2 toxin HT-2 toxin, neosolaniol and diacetoxyscirpenol (DAS) are more acutely toxic than type B trichothecenes such as deoxynivalenol (vomitoxin-DON) and nivalenol (NIV). However, NIV is present in more chronic toxicoses (Prelusky *et al.*, 1994; Rotter *et al.*, 1996).

*Fusarium poae* is considered a weak pathogen and is commonly isolated from cereal glumes (Polley & Turner, 1995). Although this species has been previously considered as a secondary pathogen in the FHB complex, recent studies have shown that *F. poae* is a more prominent FHB-causing species (Stenglein, 2009). The main type B trichothecene produced by *F. poae* is NIV, which has been found in substantial amounts in cereal samples (Schollenberger *et al.*, 2006).

The main region containing genes involved in trichothecene biosynthesis is the *TRI* gene cluster, comprising 12 genes (*tri*8, *tri*7, *tri*3, *tri*4, *tri*6, *tri*5, *tri*10, *tri*9, *tri*11, *tri*12, *tri*13 and *tri*14). Nivalenol production required *tri13* and *tri7* genes that produce the acetylation and oxygenation of the oxygen at C-4 to produce nivalenol and





FEMS MICROBIOLOGY LETTERS

4-acetyl nivalenol, respectively (Lee *et al.*, 2009). In recent years, genotype characterization based on PCR assays using primers developed from the *TRI* gene cluster to detect and screen important toxin-producing *Fusarium* species such as *Fusarium graminearum* (Chandler *et al.*, 2003; Quarta *et al.*, 2006; Ji *et al.*, 2007; Scoz *et al.*, 2009; Reynoso *et al.*, 2011; Sampietro *et al.*, 2011), *F. culmorum* (Jennings *et al.*, 2004) and *F. cerealis* (Chandler *et al.*, 2003) were published. Recently, Pasquali *et al.* (2011), comparing three PCR genotyping methods, were not able to identify NIV genotypes of *F. poae* based on the *tri7*, *tri12* and *tri13* genes, using primers previously designed for other species (Ward *et al.*, 2002; Quarta *et al.*, 2006; Wang *et al.*, 2008).

Diagnostic assays based on the PCR allow researchers to analyse the potential contamination of cereal-based food with certain mycotoxins and to determine the potential risk for human and animal health. Therefore, the aim of this study was to develop a PCR method for the detection of potential NIV-producing *F. poae* isolates.

## Materials and methods

### Fungal isolates used in this study

A total of 125 *F. poae* isolates from different countries and hosts previously identified by a species-specific PCR (Parry & Nicholson, 1996), four *F. cerealis* (NIV producers), two *F. culmorum* (NIV producers), one *F. langsethiae* (NIV producer), one *F. sporotrichioides* (NIV producer) and seven *F. graminearum* (NIV and DON producers) were analysed (Table S1, Supporting information). Moreover, NIV producers *F. austroamericanum* NRRL 2903, *F. meridionale* NRRL 28436, *F. graminearum sensu stricto* NRRL 31084 and *F. cortaderiae* NRRL 29297, from the ARS Culture Collection, and *Fusarium* species isolated from seed samples (*F. graminearum*, *F. oxysporum*, *F. chlamydosporum*, *F. sporotrichioides*, *F. equiseti* and *F. acuminatum*) were also evaluated.

### Grain samples

Twelve barley/wheat seed samples (2 kg) were provided by farmers from Buenos Aires province, Argentina. Seeds (400 per sample) were surface sterilized by immersing them for 3 min in 50% ethanol, 3 min in sodium hypochlorite (commercial 55 g Cl $L^{-1}$), washed three times with sterilized distilled water and deposited in Petri dishes (9 cm diameter) with potato dextrose agar (PDA) with chloramphenicol (50 μg $mL^{-1}$) and incubated for 7 days at 25 ± 2 °C under 12-h light/dark conditions. Potential *Fusarium* isolates were placed in tubes with PDA and in Petri dishes containing Spezieller Nährstoffarmer Agar (SNA) and incubated for 7 days at 25 ± 2 °C under 12-h light/dark conditions for the identification according to Leslie & Summerell (2006).

### DNA extraction

Monosporic genomic DNA from *Fusarium* isolates were extracted using a cetyltrimethylammonium bromide (CTAB) method described by Stenglein & Balatti (2006).

From cereal samples, 20 g of seeds per sample were ground to a fine powder for 1 min in a coffee-grinder and the DNA was extracted using the CTAB method described by Nicholson *et al.* (1996).

The quality of seed and fungal DNA was examined by electrophoresis in 0.8% (w/v) agarose gels containing GelRedTM (Biotium; Hayward) at 80 V in 1× Trisborate-EDTA buffer for 3 h at room temperature. The DNA was visualized under UV light. DNA concentrations were calculated using a fluorometer (Qubit Fluorometer; Invitrogen).

### Sequencing of the amplified products

Different set of primers (data not shown) derived from the *tri13* and the *tri7* genes of the *F. graminearum* 88-1 NIV producer (Lee *et al.*, 2001) were designed and used for a first screening (25 *F. poae* isolates selected at random). Only one primer set of the *tri7* region was able to amplify fragments of different sizes (700, 450 and 200 bp) on three *F. poae* isolates of the 25 tested. The fragments were purified by *AccuPrep*® Gel Purification Kit (Bioneer Corporation). DNA sequencing, from both the sense and antisense ends of the fragments was carried out using Big Dye Terminator version 3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, CA) in an Applied Biosystems Sequencer (ABI/Hitachi Genetic Analyzer 3130). The fragment of 450 bp was homologous to the *tri7* gene. Based on the obtained data, a specific primer pair was generated by aligning the *F. poae* sequences and the *tri7* region of the *F. graminearum* 88-1 using the Primer3 program. The selected primer sequences are nivPf (forward) 5′-TATCCTTGCATGGCAATGCC-3′ and nivPr (reverse) 5′-AAATGGCGATACGAGTATTGA-3′.

To have positive controls for the PCRs, three NIV-*F. poae* producers determined by Vogelgsang *et al.* (2008b), FP-0335, FP-0338 and FP-0378 (Table 1), plus the 17 Argentinean NIV producers determined in this study (Table 1, see Nivalenol and deoxynivalenol HPLC/FD analysis section) were used. Moreover, the fragments amplified using the NIV-*F. poae* primers of eight *F. poae* isolates selected at random (FP-TCP1a, from Argentina; FP-P2, from Canada; FP-6025, from Finland; FP-6402, 61401, and 60902, from Poland; FP-0378, from Switzerland; FP-I475, from France; Table 2) were also sequenced

**Table 1.** *Fusarium poae* isolates used as positive NIV producers

| Isolates designation | Country of origin | Year of isolation | Host | NIV production (μg $g^{-1}$) |
|---|---|---|---|---|
| FP-0335 | Switzerland | 2003 | Wheat | Vogelgsang *et al.* (2008b) |
| FP-0338 | Switzerland | 2003 | Wheat | Vogelgsang *et al.* (2008b) |
| FP-0378 | Switzerland | 2003 | Wheat | Vogelgsang *et al.* (2008b) |
| FP-HSu1a | Argentina | 2006 | Barley | 2.3 |
| FP-HTa1a | Argentina | 2007 | Barley | 0.6 |
| FP-HPu5d | Argentina | 2007 | Barley | 0.5 |
| FP-HBig1a | Argentina | 2007 | Barley | 1.0 |
| FP-TCa1a | Argentina | 2005 | Wheat | 0.4 |
| FP-TSS2b | Argentina | 2006 | Wheat | 6.1 |
| FP-THo1d | Argentina | 2006 | Wheat | 0.5 |
| FP-TPu1a | Argentina | 2005 | Wheat | 0.7 |
| FP-TSa1b | Argentina | 2007 | Wheat | 8.7 |
| FP-TBig1a | Argentina | 2007 | Wheat | 3.5 |
| FP-TMa1a | Argentina | 2007 | Wheat | 2.2 |
| FP-TPe1a | Argentina | 2007 | Wheat | 0.9 |
| FP-LSP1a | Argentina | 2003 | Tomato | 1.3 |
| FP-MICA-T-06 | Argentina | ND | Wheat | 1.5 |
| FP-MICA-A-01 | Argentina | ND | Oat | 0.3 |
| FP-MICA-A-02 | Argentina | ND | Oat | 2.6 |
| FP-MICA-A-03 | Argentina | ND | Oat | 1.9 |

ND, not determined.

to confirm that the amplified fragment corresponds to a part of the *tri7* gene sequence. The sequences were compared with the NCBI database using BLASTN (Altschul *et al.*, 1990). All sequences obtained were deposited in the NCBI/GenBank database under the accession numbers: JN614907–JN614914 (Table 2).

## PCR protocols

The PCR was carried out using 10–25 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 an XP thermal cycler (Bioer Technology Co.) with an initial denaturing step at 95 °C for 2 min, followed by 25 cycles at 95 °C for 10 s (denaturing step), 65 °C for 10 s (annealing), 72 °C for 20 s (extension) and a final extension cycle at 72 °C for 2 min.

PCRs using available species-specific primers for the *Fusarium* species isolated from grains (*F. graminearum*, *F. acuminatum*, *F. oxysporum*, *F. sporotrichioides* and *F. equiseti*) were made. The PCRs were carried out as described above, but using specific annealing temperatures and cycles according to Nicholson *et al.* (1998), Williams *et al.* (2002), Mishra *et al.* (2003), Niessen *et al.* (2004) and Jurado *et al.* (2005).

Products from PCRs were examined by electrophoresis in 1.5% (w/v) agarose gels containing GelRedTM (Biotium; Hayward) at 80 V in 1× Trisborate-EDTA buffer for 1 h at room temperature. Fragments were visualized under UV light. The size of the DNA fragments was estimated by comparing the DNA bands with a 100 bp DNA ladder (Genbiotech S.R.L., Buenos Aires, Argentina).

**Table 2.** *Fusarium poae* isolates used for sequencing

| Isolates designation | Country of origin | Year of isolation | Host | Accession number |
|---|---|---|---|---|
| FP-TCP1a | Argentina | 2004 | Wheat | JN614914 |
| FP-P2 | Canada | 2008 | ND | JN614913 |
| FP-6025 | Finland | 2006 | Barley | JN614907 |
| FP-6402 | Poland | 2006 | Wheat | JN614908 |
| FP-61401 | Poland | 2006 | Wheat | JN614910 |
| FP-60902 | Poland | 2006 | Wheat | JN614909 |
| FP-0378′ | Switzerland | 2003 | Wheat | JN614911 |
| FP-I475 | France | 2007 | Wheat | JN614912 |

ND, not determined.

## Nivalenol and deoxynivalenol HPLC/FD analysis

Seventeen Argentinean *F. poae* isolates from different regions and hosts selected at random were analysed by HPLC/FD to test NIV/DON production (Table 1). *Fusarium poae* isolates were cultured in Erlenmeyer flasks (250 mL) containing 25 g of long-grain rice. Ten mL of distilled water was added before autoclaving for 30 min at 121 °C, twice. Each flask was inoculated with a 3-mm diameter agar disc taken from the margin of a colony

grown on SNA (Nirenberg, 1976) at 25 °C for 7 days. Flasks were shaken once a day by hand for 1 week. These cultures were incubated for 28 days at 25 °C in the dark. At the end of the incubation period, the contents of the flask were dried at 50 °C for 24 h and then stored at −20 °C until being analysed for toxin.

Toxin extraction and clean-up were carried out using a modified version of that originally reported by Cooney *et al.* (2001). For the detection of NIV and DON, the analysis was performed using the conditions described by Barros *et al.* (2008). The dried residue was dissolved in 400 μL of methanol/water (5 : 95), homogenized in a vortex mixer and injected into the HPLC system by full-loop injection technique (Hewlett Packard model 1100 pump, Palo Alto, CA and Rheodyne manual injector with a 50 μL loop; Rheodyne, Cotati, CA). The HPLC system consisted of a Hewlett Packard model 1100 pump connected to a Hewlett Packard 1100 Series variable wavelength detector and a data module Hewlett Packard Kayak XA (HP ChemStation Rev. A.06.01). Chromatographic separations were performed on a Luna$^{TM}$ C18 reversed-phase column (100 × 4.6 mm, 5 μM particle size) connected to a guard column SecurityGuard$^{TM}$ (4 × 3.0 mm) filled with the same phase. The mobile phase consisted of methanol/water (12 : 88), at a flow rate of 1.5 mL min$^{-1}$. The detector was set at 220 nm with an attenuation of 0.01 AUFS. Quantification was relative to external standards of DON and NIV (Sigma-Aldrich Co., St Louis, MO) from 1 to 4 μg mL$^{-1}$ in methanol/water (5 : 95). The detection limit was 5 ng g$^{-1}$ for each toxin.

## Results and discussion

*Fusarium poae* is recognized as a more prominent member of the FHB complex (Yli-Mattila *et al.*, 2008; Kulik & Jestoi, 2009; Stenglein, 2009). Several researchers have developed specific primer pairs for PCR assays, to have a rapid, inexpensive and relative simple technique to identify *F. poae* isolates of cereal samples (Parry & Nicholson, 1996; Kulik, 2008; Yli-Mattila *et al.*, 2008). *Fusarium poae* isolates used in our study were found to be positive based on the specific primer pair developed by Parry & Nicholson (1996).

Seventeen Argentinean isolates were analysed by HPLC/FD for production of trichothecenes and only NIV was detected (0.3–8.7 μg g$^{-1}$; Table 1). This was in agreement with other studies (Vogelgsang *et al.*, 2008a, b; Yli-Mattila *et al.*, 2008), although a small number of isolates of the fungus have been reported that do not produce the toxin (Thrane *et al.*, 2004; Somma *et al.*, 2010). Variations in the produced toxin levels in the literature can be explained by differences in extraction or culturing of the isolates (Vogelgsang *et al.*, 2008a; Kokkonen *et al.*, 2010; Fanelli *et al.*, 2012).

Sequenced fragments of eight *F. poae* isolates were very homologous (99–100%) and showed 81% homology with the *tri7* gene (E-value 1e−57) of *Fusarium graminearum* 88-1.

Several studies have been carried out to detect natural contamination of cereals and grain-based products with mycotoxins producing species of the FHB complex using PCR assays. Lee *et al.* (2001) identified genetic differences between the trichothecene biosynthetic pathways of the NIV and DON chemotypes and developed a rapid method for *Gibberella zeae* genotype identification based on PCR analysis. Ward *et al.* (2002) designed specific primers based on the *tri12* gene sequences to identify NIV-producing *F. graminearum* isolates. Chandler *et al.* (2003) developed a number of PCR assays to amplify *tri7* and *tri13* sequences to characterize isolates of *F. graminearum, F. culmorum* and *F. cerealis* in terms of their NIV and DON potential production. Quarta *et al.* (2005) were able to develop specific primers targeting the *tri3* and *tri7* genes to identify 3A-DON, 15A-DON and NIV-*F. culmorum* producers based on the sequences of *Fusarium graminearum* described by Lee *et al.* (2001) and Ward *et al.* (2002). In our study, the PCR program was adjusted to different annealing temperatures and the number of cycles was reduced to obtain a rapid and reliable technique. The selected primers were evaluated on genomic DNA extracted from 125 *F. poae* isolates from

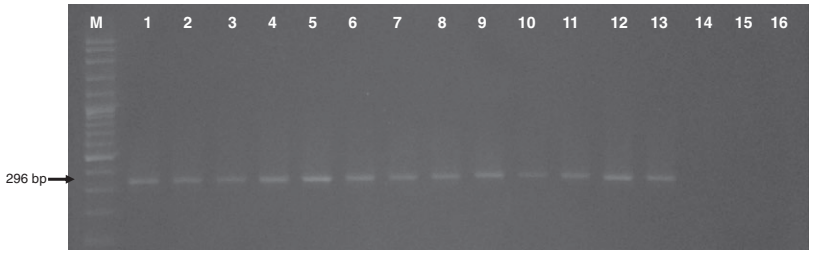


**Fig. 1.** Amplification of the specific fragment to detect nivalenol-producing *Fusarium poae* isolates. Lanes: M, 100 bp ladder; from 1 to 13, *Fusarium poae* isolates; 14, *F. culmorum*; 15, *F. sporotrichioides*; 16, *F. langsethiae*.

13 different countries and eight different hosts, plus other *Fusarium* species tested (see Materials and methods section). The *F. poae* isolates showed the presence of the 296-bp partial *tri7* DNA fragment (Fig. 1), whereas no product was amplified from other *Fusarium* species.

In our cereal sample analyses, *Fusarium poae* was the species with higher isolation frequency (15 isolates) in all seed samples analysed, followed by *F. graminearum* (seven), *F. oxysporum* (four), *F. chlamydosporum* (three), *F. acuminatum* (one), *F. equiseti* (one) and *F. sporotrichioides* (one). All of these isolates were tested with the new primer set for potential NIV-*F. poae* producers and only *F. poae* isolates amplified the expected fragment. Moreover, DNA obtained from seed samples amplified the product of 296 bp according to the size of our NIV-*F. poae*-specific PCR.

## Acknowledgements

This work was supported by FONCYT-SECYT PRH32-PICT 2008/110 and PIP 167 CONICET.

## References

Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ (1990) Basic local alignment search tool. *J Mol Evol* **215**: 403–410.

Barros G, García D, Oviedo S, Ramirez ML, Torres A & Chulze S (2008) Deoxynivalenol and nivalenol analysis in soybean and soy flour. *World Mycotoxin J* **1**: 263–266.

Chandler EA, Simpson DR, Thomsett MA & Nicholson P (2003) Development of PCR assays to *Tri7* and *Tri13* trichothecene biosynthetic genes, and characterisation of chemotypes of *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium cerealis*. *Physiol Mol Plant Pathol* **62**: 355–367.

Cooney JM, Lauren DR & di Menna ME (2001) Impact of competitive fungi on trichothecene production by *Fusarium graminearum*. *J Agric Food Chem* **49**: 522–526.

Desjardins AE (2006) *Fusarium Mycotoxins Chemistry Genetics and Biology*. APS Press, St. Paul, MN.

Fanelli F, Schmidt-Heydt M, Haidukowski M, Geisen R, Logrieco A & Mulé G (2012) Influence of light on growth, fumonisin biosynthesis and *FUM1* gene expression by *Fusarium proliferatum*. *Int J Food Microbiol* **153**: 148–153.

Jennings P, Coates ME, Turner JA, Chandler EA & Nicholson P (2004) Determination of deoxynivalenol and nivalenol chemotypes of *Fusarium culmorum* isolates from England and Wales by PCR assay. *Plant Pathol* **53**: 182–190.

Ji L, Cao K, Hu T & Wang S (2007) Determination of deoxynivalenol and nivalenol chemotypes of *Fusarium graminearum* isolates from China by PCR assay. *J Phytopathol* **155**: 505–512.

Jurado M, Vázquez C, Patiño B & Gonzalez-Jaén MT (2005) PCR detection assays for the trichothecene-producing species *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium poae*, *Fusarium equiseti* and *Fusarium sporotrichioides*. *Syst Appl Microbiol* **28**: 562–568.

Kokkonen M, Ojala L, Parikka P & Jestoi M (2010) Mycotoxin production of selected *Fusarium* species at different culture conditions. *Int J Food Microbiol* **143**: 17–25.

Kulik T (2008) Development of a duplex PCR assay for the simultaneous detection of *Fusarium poae* and *Fusarium sporotrichioides* from wheat. *J Plant Pathol* **90**: 425–432.

Kulik T & Jestoi M (2009) Quantification of *Fusarium poae* DNA and associated mycotoxins in asymptomatically contaminated wheat. *Int J Food Microbiol* **130**: 233–237.

Kulik T, Pszczólkowska A, Fordonski G & Olszewski J (2007) PCR approach based on the *esyn1* gene for the detection of potential enniatin-producing *Fusarium* species. *Int J Food Microbiol* **116**: 319–324.

Lee T, Oh D-W, Kim H-S, Lee J, Kim Y-H, Yun S-H & Lee Y-W (2001) Identification of deoxynivalenol- and nivalenol-producing chemotypes of *Gibberella zeae* using PCR. *Appl Environ Microbiol* **67**: 2966–2972.

Lee J, Chang I-Y, Kim H, Yun S-H, Leslie JF & Lee Y-W (2009) Genetic diversity and fitness of *Fusarium graminearum* populations from rice in Korea. *Appl Environ Microbiol* **75**: 3289–3295.

Leslie JF & Summerell BA (2006) *The Fusarium Laboratory Manual*. Blackwell Publishing, Ames, IA.

Mishra PK, Fox RTV & Culham A (2003) Development of a PCR-based assay for rapid and reliable identification of pathogenic Fusaria. *FEMS Microbiol Lett* **218**: 329–332.

Moreno EC, Garcia GT, Ono MA, Vizoni É, Kawamura O, Hirooka EY & Ono EYS (2009) Co-ocurrence of mycotoxins in corn samples from the Northern region of Paraná State, Brazil. *Food Chem* **116**: 220–226.

Nicholson P, Lees AK, Maurin N, Parry DW & Rezanoor HN (1996) Development of a PCR assay to identify and quantify *Microdochium nivale* var. *nivale* and *Microdochium nivale* var. *majus* in wheat. *Physiol Mol Plant Pathol* **48**: 257–271.

Nicholson P, Simpson DR, Weston G, Rezanoor HN, Lees AK, Parry DW & Joyce D (1998) Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. *Physiol Mol Plant Pathol* **53**: 17–37.

Niessen L, Schmidt H & Vogel RF (2004) The use of *Tri5* gene sequences for PCR detection and taxonomy of trichothecene-producing species in the *Fusarium* section *Sporotrichiella*. *Int J Food Microbiol* **95**: 305–319.

Nirenberg HI (1976) Untersuchungen über die morphologische und biologisch Diffrenzieerum in der *Fusarium* Sekion Lisiola. *Mitt Biol Bundesanst Land-Forstwirtsch* **169**: 1–117.

Parry DW & Nicholson P (1996) Development of a PCR assay to detect *Fusarium poae* in wheat. *Plant Pathol* **45**: 383–391.

Pasquali M, Beyer M, Bohn T & Hoffmann L (2011) Comparative analysis of genetic chemotyping methods for *Fusarium: Tri13* polymorphism does not discriminate between 3- and 15-acetylated deoxynivalenol chemotypes in *Fusarium graminearum*. *J Phytopathol* **159**: 700–704.

Polley RW & Turner JA (1995) Surveys of stem base diseases and *Fusarium* ear diseases in winter wheat in England, Wales and Scotland, 1989-1990. *Ann Appl Biol* **126**: 45–59.

Prelusky DB, Rotter BA & Rotter RG (1994) Toxicology of mycotoxins. *Mycotoxins in Grain: Compounds other than Aflatoxin* (Miller JD & Trenholm HL, eds), pp. 359–404. Eagan Press, St. Paul, MN.

Quarta A, Mita G, Haidukowski M, Santino A, Mulé G & Visconti A (2005) Assessment of trichothecene chemotypes of *Fusarium culmorum* occurring in Europe. *Food Addit Contam* **22**: 309–315.

Quarta A, Mita G, Haidukowski M, Logrieco A, Mulé G & Visconti A (2006) Multiplex PCR assay for the identification of nivalenol 3- and 15-acetyl-deoxynivalenol chemotypes in *Fusarium*. *FEMS Microbiol Lett* **259**: 7–13.

Reynoso MM, Ramirez ML, Torres AM & Chulze SN (2011) Trichothecene genotypes and chemotypes in *Fusarium graminearum* strains isolated from wheat in Argentina. *Int J Food Microbiol* **145**: 444–448.

Rotter B, Prelusky DB & Petska JJ (1996) Toxicology of deoxynivalenol (vomitoxin). *J Toxicol Environ Health* **48**: 1–34.

Sampietro DA, Diaz CG, Gonzalez V, Vattuone MA, Ploper LD, Catalan CA & Ward TJ (2011) Species diversity and toxigenic potential of *Fusarium graminearum* complex isolates from maize fields in northwest Argentina. *Int J Food Microbiol* **145**: 359–364.

Schollenberger M, Müller H-M, Rüfle M, Suchy S, Plank S & Drochner W (2006) Natural ocurrence of 16 *Fusarium* toxins in grains and feedstuffs of plant origin from Germany. *Mycopathologia* **161**: 43–52.

Scoz LB, Astolfi P, Reartes DS, Schmale DG, Moraes MG & Del Ponte EM (2009) Trichothecene mycotoxin genotypes of *Fusarium graminearum sensu stricto* and *Fusarium meridionale* in wheat from southern Brazil. *Plant Pathol* **58**: 344–351.

Somma S, Alvarez C, Ricci V, Ferracane L, Ritieni A, Logrieco A & Moretti A (2010) Trichothecene and beauvericin mycotoxin production and genetic variability in *Fusarium poae* isolated from wheat kernels from northern Italy. *Food Addit Contam* **27**: 729–737.

Stenglein SA (2009) *Fusarium poae:* a pathogen that needs more attention. *J Plant Pathol* **91**: 25–36.

Stenglein SA & Balatti PA (2006) Genetic diversity of *Phaeoisariopsis griseola* in Argentina as revealed by pathogenic and molecular markers. *Physiol Mol Plant Pathol* **68**: 158–167.

Thrane U, Adler A, Clasen P-E, Galvano F, Langseth W, Lew H, Logrieco A, Nielsen KF & Ritieni A (2004) Diversity in metabolite production by *Fusarium langsethiae*, *Fusarium poae*, and *Fusarium sporotrichioides*. *Int J Food Microbiol* **95**: 257–266.

Vogelgsang S, Sulyok M, Bänziger I, Krska R, Schuhmacher R & Forrer H-R (2008a) Effect of fungal strain and cereal substrate on *in vitro* mycotoxin production by *Fusarium poae* and *Fusarium avenaceum*. *Food Addit Contam* **25**: 745–757.

Vogelgsang S, Sulyok M, Hecker A, Jenny E, Krska R, Schuhmacher R & Forrer H-R (2008b) Toxigenicity and pathogenicity of *Fusarium poae* and *Fusarium avenaceum* on wheat. *Eur J Plant Pathol* **122**: 265–276.

Wang J-H, Li H-P, Qu B, Zhang J-B, Huang T, Chen F-F & Liao Y-C (2008) Development of a generic PCR detection of 3-acetyldeoxynivalenol- 15-acetyldeoxynivalenol- and nivalenol chemotypes of *Fusarium graminearum* clade. *Int J Mol Sci* **9**: 2495–2504.

Ward TJ, Bielawski JP, Kistler HC, Sullivan E & O'Donnell K (2002) Ancestral polymorphism and adaptative evolution in the trichothecene mycotoxin gene cluster of phytopathogenic *Fusarium*. *P Natl Acad Sci USA* **99**: 9278–9283.

Williams KJ, Dennis JT, Smyl C & Wallwork H (2002) The application of species-specific assays based on the polymerase chain reaction to analyze *Fusarium* crown rot of durum wheat. *Australas Plant Pathol* **31**: 119–127.

Yli-Mattila T, Paavanen-Huhtala S, Jestoi M, Parikka P, Hietaniemi V, Gagkaeva T, Sarlin T, Haikara A, Laaksonen S & Rizzo A (2008) Real-time PCR detection and quantification of *Fusarium poae*, *F. graminearum*, *F. sporotrichioides* and *F. langsethiae* in cereal grains in Finland and Russia. *Arch Phytopathology Plant Protect* **41**: 243–260.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** *Fusarium* isolates used in this study for specific NIV-PCR analysis.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.