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SHORT COMMUNICATION

Fusarium cerealis Associated with Barley Seeds in Argentina

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Abstract

Fusarium head blight is a fungal disease caused by a complex of *Fusarium* species on cereals, such as barley and wheat. It has economic impacts due to yield reductions and mycotoxin contamination. As barley production has increased considerably in the last 5 years in Argentina, a survey was conducted for identifying *Fusarium* species associated with barley grains. *Fusarium cerealis* was isolated and identified based on morphological and molecular analysis. The potential production of nivalenol and zearalenone was assessed using specific PCR assays. Koch's postulates were carried out to confirm the pathogenicity of the fungus.

Introduction

Fusarium head blight (FHB) is a major disease of wheat, barley and other small grain cereals, caused by a complex of *Fusarium* species. This disease reduces grain yield and can also produce (depending on the associated *Fusarium* species) different mycotoxins, especially trichothecenes, a group that can cause a wide range of acute and chronic effects in humans and animals (D'Mello et al. 1999). Deoxynivalenol (DON) and nivalenol (NIV) are considered the most widespread type B trichothecenes produced by *Fusarium* species isolated from small grain cereals. DON and NIV can be produced by several *Fusarium* species, such as *Fusarium graminearum*, *F. culmorum*, *F. poae* and *F. cerealis*, among others (Desjardins 2006). Zearalenone (ZEA) is a polyketide mycotoxin produced by some *Fusarium* species that has an oestrogenic effect on mammals affecting their reproductive systems (Desjardins 2006).

Barley (*Hordeum vulgare* L.) is one of the most widely grown winter cereal crops in Argentina (1.5 M ha in 2012) and is primarily grown for use as malted barley. However, barley grain of insufficient quality for the brewing process is used as source of energy and protein for farm livestock.

Fusarium head blight symptoms on barley include premature necrosis and a brown/grey discolouration of the spike (Boddu et al. 2006), and the presence of *Fusarium* species in barley grain poses a risk to consumers because of the associated mycotoxins.

Fusarium cerealis (synonym of *F. crookwellense*, see www.mycobank.org) has been isolated from wheat-growing areas at low frequencies (Gale 2003; Alvarez et al. 2011; Schmale et al. 2011). However, less extensive surveys of the incidence of *Fusarium* spp. in barley grains have been undertaken to date. To our knowledge, little is known (Barreto et al. 2004; Castañares et al. 2012) about the occurrence and distribution of *Fusarium* species in barley grains in Argentina; hence, the aim of this study was to report the occurrence of *F. cerealis* on barley grains based on both morphological and molecular methods. As *F. cerealis* was reported to produce NIV and ZEA (di Menna et al. 1991; Miller et al. 1991), the potential ability to produce these toxins was tested using specific PCR assays.

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Material and Methods

A survey of fungi in a barley (cv. Shakira) seed lot from a field located at Treinta de Agosto (36°16′0″ S, 62°33′0″ W), Buenos Aires, Argentina, in the 2011 growing season, was conducted. Four-hundred seeds were surface sterilized (70% EtOH for 2 min; 5% NaClO for 2 min), rinsed twice in sterilized distilled water, plated on 2% potato dextrose agar (PDA) and incubated at 24 ± 2°C in a 12-h dark/light cycle. Ten fungal colonies morphologically similar to *Fusarium* were observed after 6 days of incubation. For identification, all isolates were taken and purified through monosporic culture and transferred onto PDA and carnation leaf agar (CLA) to grow in the same conditions as described above. Identification to species based on morphological traits was carried out for all the isolates according to Leslie and Summerell (2006).

For Koch's postulates, a pathogenicity test was conducted using a hand sprayer to inoculate 30 barley (cv. Shakira) heads selected at random in potted plants (one plant per pot with ±5 heads per plant) with a 5-ml spore suspension (8×10^4 conidia/ml). Ten heads selected at random from different potted barley plants were sprayed with sterile distilled water as a control. The whole plants were covered with polyethylene bags and incubated for 3 days in a growth chamber under a 12-h light/dark cycle at 22 ± 2°C. Plants were unbagged and moved into a greenhouse. Twenty days after inoculation, the spikelets were visually evaluated for disease symptoms.

DNA of the monosporic fungus morphologically identified as *F. cerealis* was extracted as described previously (Stenglein and Balatti 2006) and identified to species level by sequencing portions of the reductase (*RED*), trichothecene 3-*O*-acetyltransferase (*tri101*) and translation elongation factor (*TEF*) genes (Ward et al. 2008). PCR products were purified with the aid of a PureLink PCR purification kit (Invitrogen, Löhne, Germany). 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, Foster City, CA, USA) in an Applied Biosystems Sequencer (ABI/Hitachi Genetic Analyzer 3130). Similarities of the fragments with previously published sequences were examined with BLASTN (Altschul et al. 1990) on the NCBI website. The sequences generated in this study were submitted to GenBank.

Additionally, the isolate was tested for the potential to produce NIV based on the *Tri7* gene and ZEA based on the *PKS4* gene, using specific PCR primers according to Quarta et al. (2006) and Meng et al. (2010), respectively.

All PCRs were carried out in a 25-μl final volume containing 12–15 ng of genomic DNA, 10× reaction buffer [2 mM Tris–HCl pH 8.0, 10 mM KCl, 0.01 mM EDTA, 1 mM DTT, 50% (v/v) glycerol, 0.5% (v/v) Tween® 20, 0.5% (v/v) Nonidet®P40], 0.5 μM of each primer, 200 μM of each dNTP (Genbiotech S.R.L., Buenos Aires, Argentina), 2.5 mM MgCl2 and 1.25 units of Taq DNA polymerase (InbioHighway, Tandil, Argentina). DNA amplifications were performed in an XP thermal cycler (Bioer Technology Co, Hangzhou, China) with an initial denaturing step at 95°C for 2 min; followed by 29 cycles at 95°C for 30 s, 57°C (for *RED* and *tri101* primers) for 35 s and 72°C for 45 s; and a final extension cycle at 72°C for 2 min. The annealing temperature was modified according to the primers used: 54°C (*TEF*), 53°C (NIV) and 60°C (ZEA). Each reaction was performed twice. Products were examined by electrophoresis in 1.5% (w/v) agarose gels containing GelRed™ (Biotium, Hayward, CA, USA) at 80 V in 1× Trisborate–EDTA buffer for 2 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.). Gel images were photographed with a digital DOC 6490 system (Biodynamics S.R.L., Buenos Aires, Argentina).

Results and Discussion

One isolate, when growing on PDA, produced abundant white aerial mycelium and formed red pigments in the medium. Abundant sporodochia were observed. On CLA, macroconidia were abundant and formed monophialides in pale orange sporodochia, falcate with the dorsal side more curved than the ven-

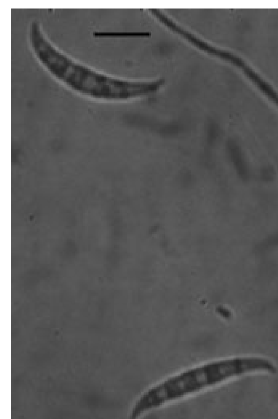

Fig. 1 *Fusarium cerealis* macroconidia. Scale bar 25 μm.

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tral, commonly five septate (Fig. 1). Microconidia were not observed. Chlamydospores in aerial hyphae and in macroconidia were observed after 4 weeks in chains and clusters. The fungus was identified as *F. cerealis* (Cooke) Sacc. (synonym *F. crookwellense* Burgess, Nelson & Toussoun) on the basis of morphological characters.

Twenty days after inoculation, control spikelets were asymptomatic, and inoculated spikelets showed discoloration and/or a tan to dark brown necrosis (Fig. 2). Portions of the rachis were brown to dark brown. The fungus was re-isolated from symptomatic kernels and identified based on morphological traits, demonstrating the pathogenicity on barley spikes.

The *RED* (accession no. JX682529), *tri101* (accession no. JX682530) and *TEF* (accession no. JX682531) sequences showed 100% similarity with several *F. cerealis* sequences, confirming the morphological identification (Table 1).

Fusarium cerealis was first isolated from potato tubers in Australia in 1971 (Burgess et al. 1982) and is frequently found in temperate regions being reported in Australia, Canada, Japan, New Zealand, South Africa, the USA and different parts of Europe (Chandler et al. 2003; Lauren et al. 1992; Leslie and Summerell 2006; Schmale et al. 2011; Sugiura et al. 1993). López et al. (1997) isolated *F. cerealis* from maize, and Alvarez et al. (2011) recovered two isolates of this *Fusarium* species from wheat grains in Argentina. Moreover, Zhang et al. (2011) reported the first occurrence of *F. cerealis* causing FHB on barley in China.

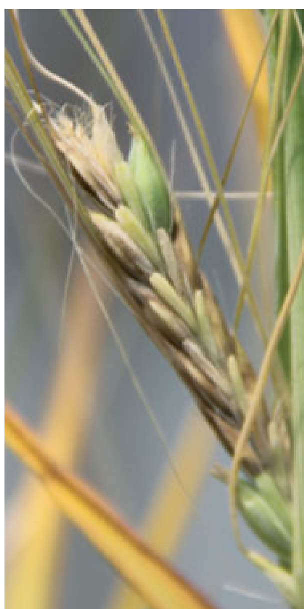

Fig. 2 *Fusarium cerealis* symptoms on barley.

Table 1 *Fusarium cerealis* sequences identity compared with published GenBank accessions

F. cerealis sequence	*e*-Value	Identity (%)	GenBank accession no.
RED	0.0	100	EF428900
JX682529	0.0	100	DQ382043
	0.0	100	AF212576
tri101	0.0	100	AF212612
JX682530	0.0	100	HQ149735
	0.0	100	JF911476
TEF	0.0	100	GQ915505
JX682531	0.0	100	FJ939661
	0.0	99	FJ939660

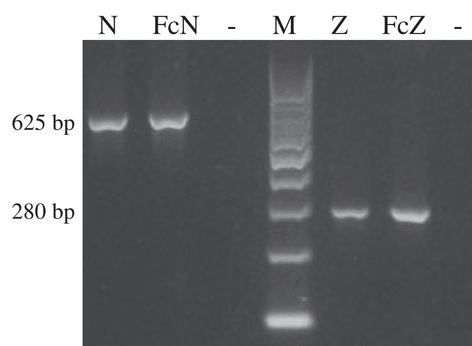

Fig. 3 Amplification of the NIV- and ZEA-specific fragments of *Fusarium cerealis*. (N) positive NIV control (*F. austroamericanum* NRRL 2903), (FcN) *F. cerealis* NIV reaction, (–) negative control, (M) molecular 100-bp marker, (Z) positive ZEA control (*F. graminearum sensu stricto* NRRL 31084), (FcZ) *F. cerealis* ZEA reaction, (–) negative control. NRRL isolates were kindly provided by the ARS Culture Collection.

Fusarium cerealis is considered a minor component of the FHB species complex with the ability to produce NIV and ZEA *in vitro* (di Menna et al. 1991; Miller et al. 1991) and was suggested to be one of the *Fusarium* species responsible for NIV/ZEA contamination of wheat grains in Hokkaido, Japan (Sugiura et al. 1993). Our results based on PCR assays confirmed the potential of this species to produce NIV and ZEA based on the amplification of a 625- and 280-bp product (Fig. 3), respectively (Quarta et al. 2006; Meng et al. 2010).

To our knowledge, this is the first report of *F. cerealis* associated with barley kernels in Argentina. Considering the increasing importance of barley in Argentina and the potential of this fungus to cause head blight and mycotoxins, a large-scale survey of *F. cerealis* on barley crops in Argentina is underway.

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References

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

Alvarez CL, Somma S, Proctor RH, Stea G, Mulé G, Logrieco AF, Fernandez Pinto V, Moretti A. (2011) Genetic diversity in *Fusarium graminearum* from a major wheat-producing region of Argentina. Toxins 3:1249–1309.

Barreto D, Carmona M, Ferrazini M, Zanelli M, Perez BA. (2004) Occurrence and pathogenicity of *Fusarium poae* in barley in Argentina. Cereal Res Commun 32:53–60.

Boddu J, Cho S, Kruger WM, Muehlbauer GJ. (2006) Transcriptome analysis of the barley-*Fusarium graminearum* interaction. Mol Plant Microbe Interact 19:407–417.

Burgess LW, Nelson PE, Toussoun TA. (1982) Characterization, geographic distribution, and ecology of *Fusarium crookwellense sp. nov*. Trans Brit Mycol Soc 79:497–505.

Castañares E, Wehrhahne L, Stenglein SA. (2012) *Fusarium pseudograminearum* associated with barley kernels in Argentina. Plant Dis 96:763.

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

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

D'Mello JPF, Placinta CM, McDonald AMC. (1999) *Fusarium* mycotoxins: a review of global implications for animal health, welfare and productivity. Anim Feed Sci Technol 80:183–205.

Gale LR. (2003) Population biology of *Fusarium* species causing head Blight of grain crops. In: Leonard KJ, Bushnell WR (eds) Fusarium Head Blight of Wheat and Barley. St Paul, MN, USA, APS Press, pp 120–143.

Lauren DR, Sayer ST, Menna ME. (1992) Trichothecene production by *Fusarium* species isolated from grain and pasture throughout New Zealand. Mycopathologia 120:167–176.

Leslie JF, Summerell BA. (2006) The Fusarium Laboratory Manual. Oxford, UK, Blackwell Publishing.

López TA, Escande A, Chayer R, Dosanto M, Gerpe O, Salomón ML. (1997) *Fusarium crookwellense* produced zearalenone in maize stubble in the field. N Z Vet J 45:251–253.

Meng K, Wang Y, Yang P, Luo H, Bai Y, Shi P, Yuan T, Ma R, Yao B. (2010) Rapid detection and quantification of zearalenone-producing *Fusarium* species by targeting the zearalenone synthase gene *PKS4*. Food Control 21:207–211.

di Menna ME, Lauren DR, Smith WA. (1991) Effect of incubation temperature on zearalenone production by strains of *Fusarium crookwellense*. Mycopathologia 116:81–86.

Miller JD, Greenhalgh Y, Wang Y, Lu M. (1991) Trichothecene chemotypes of three *Fusarium* species. Mycologia 83:121–130.

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

Schmale DG, Wood-Jones AK, Cowger C, Bergstrom GC, Arellano C. (2011) Trichothecene genotypes of *Gibberella zeae* from winter wheat fields in the eastern USA. Plant Pathol 60:909–917.

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

Sugiura Y, Fukasaku K, Tanaka T, Matsui Y, Ueno Y. (1993) *Fusarium poae* and *Fusarium crookwellense*, fungi responsible for the natural occurrence of nivalenol in Hokkaido. Appl Environ Microbiol 59:3334–3338.

Ward TJ, Clear RM, Rooney AP, O'Donnell K, Gaba D, Patrick S, Starkey DE, Gilbert J, Geiser DM, Nowicki TW. (2008) An adaptative evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Gen Biol 45:473–484.

Zhang H, Chen WQ, Xu J, Xu JS, Feng J. (2011) First report of *Fusarium cerealis* causing Fusarium head blight on barley in China. Plant Dis 95:774.

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