Food Chemistry 196 (2016) 445–450

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Fungal diversity and natural occurrence of deoxynivalenol and zearalenone in freshly harvested wheat grains from Brazil

Sabina Moser Tralamazza [a,*], Renata Heidtmann Bemvenuti [b], Patrícia Zorzete [a], Fábio de Souza Garcia [a], Benedito Corrêa [a]

[a] Laboratory of Mycotoxins and Toxigenic Fungi, Institute of Biomedical Sciences, University of Sao Paulo, 05508-000 Sao Paulo, Brazil
[b] Laboratory of Food Science and Mycotoxins, Federal University of Rio Grande, 96201-900 Rio Grande, Brazil

ARTICLE INFO

Article history:
Received 31 January 2015
Received in revised form 14 September 2015
Accepted 16 September 2015
Available online 21 September 2015

Keywords:
Fusarium
Wheat
Mycobiota
Deoxynivalenol
Zearalenone

ABSTRACT

This study investigated the fungal diversity and presence of deoxynivalenol and zearalenone in 150 samples of freshly harvested wheat grains collected in three regions of Brazil (Sao Paulo, Parana, and Rio Grande do Sul). Analysis of the mycobiota showed a predominance of *Alternaria* sp., *Fusarium* sp. and *Epicoccum* sp. *Microdochium nivale* (23%), a fungus rarely found in Brazilian crops, was detected in Sao Paulo. Four members of the *Fusarium graminearum* species complex were isolated: *F. graminearum s.s.* (37%), *Fusarium meridionale* (46%), *Fusarium cortaderiae* (13%), and *Fusarium austroamericanum* (3%). Toxin analysis revealed 99% contamination with deoxynivalenol (mean 706 μg/kg). The frequency of zearalenone varied greatly across regions: wheat grains from Rio Grande do Sul (84%) and Sao Paulo (12%) had median concentrations of 70.9 and 57.9 μg/kg, respectively. ZEA was not detected in the samples from Parana. A total of six samples were above the maximum tolerated level recommended by the European Commission for ZEA in wheat grains. This study provided new insights into the natural mycobiota of Brazilian wheat, demonstrating contamination of most samples with deoxynivalenol and high frequency of zearalenone in samples from Rio Grande do Sul.

1. Introduction

A large portion of wheat in Brazil is imported. However, excellent grain yields and a significant increase in crop area (CONAB Companhia Nacional de Abastecimento., 2013) are gradually increasing the role of national wheat cultivation. In this respect, the investigation of the microbiology and mycotoxigenic species in wheat grain is important to produce and maintain a quality product for commercialization.

Fusarium head blight (FHB) is a disease of cereal crops that can cause extensive losses in crop production and yield and affect prices due to the presence of *Fusarium*-damaged kernels. Additionally, FHB is associated with the presence of several mycotoxins, particularly trichothecenes and zearalenone (ZEA). The disease is mainly caused by members of the *Fusarium graminearum* species complex (FGSC), but other pathogens such as *Fusarium culmorum*, *Fusarium avenaceum* and *Microdochium nivale* have also been reported (O'Donnell, Ward, Geiser, Corby Kistler, & Aoki, 2004; Wiese, 1987).

Deoxynivalenol (DON), which belongs to the trichothecene group, is a stable compound during storage and processing of food and is very resistant to high temperatures (Wolf-Hall, Hanna, & Bullerman, 1999). In animals, the ingestion of DON can cause nutritional deficiencies, vomiting, weight loss, and anorexia (Rotter, Prelusky, & Pestka, 1996; Smith, McMillan, & Castillo, 1997). ZEA is another compound produced by members of the FGSC. In view of its high affinity for estrogen receptors, this compound can cause significant changes in reproductive organs and fertility loss in animals and humans (Zinedine, Soriano, Moltó, & Mañes, 2007). All members of the FGSC are able to produce at least one type of trichothecene and the vast majority also produces ZEA (O'Donnell, Kistler, Tacke, & Casper, 2000). Trichothecene chemotype profiling is used to classify the potential of fungi to produce a variety of trichothecenes type B. Fungi of the nivalenol (NIV) chemotype produce NIV and NIV derivatives (4-acetylnivalenol). Strains of the 3-acetyldeoxynivalenol (3ADON) or 15-acetyldeoxynivalenol (15ADON) chemotype produce DON and either 3ADON or 15ADON (Desjardins, 2006). Chemotype profiling is important because species of the FGSC and their ability to produce trichothecenes vary

* Corresponding author at: Av. Prof. Lineu Prestes, 1374, CEP 05508-000 São Paulo, Brazil.
E-mail address: sabina@usp.br (S.M. Tralamazza).

http://dx.doi.org/10.1016/j.foodchem.2015.09.063
0308-8146/

worldwide. In most part of South and North America, the main causal agent of FHB is *F. graminearum sensu stricto (s.s.)* (Pan, Calero, Mionetto, & Bettucci, 2013; Ramirez et al., 2007; Ward et al., 2008).

In view of the scarcity of data on the mycobiota and toxin content of wheat grains, particularly ZEA, the objective of the present study was to evaluate fungal diversity by molecular analysis and the presence of DON and ZEA in freshly harvested wheat grains in Brazil.

2. Materials and methods

2.1. Wheat samples and water activity

In this study, 150 samples of freshly harvested wheat grains were collected in three different regions of Brazil (50 samples/region) between September and November of the 2012 harvest: Novo Itacolomi (Parana State, PR), Passo Fundo (Rio Grande do Sul State, RS), and Capao Bonito (Sao Paulo State, SP) (Fig. 1). Grains were collected 3–6 days after harvest. Samples of approximately 1 kg each were collected and stored at 4 °C for immediate analysis of the mycobiota. Water activity (A_w) was determined with an Aqualab CX-2 apparatus. After fungal isolation, the samples were stored at −20 °C until the time of mycotoxin analysis.

2.2. Fungal isolation

For fungal isolation, subsamples (100 g) of the wheat grains were disinfected with commercial sodium hypochlorite solution (1%) for 1 min and washed two times with distilled water. Subsamples (100 grains) were placed on PDA plates (10 grains/plate) and incubated for 5 days at 25 °C.

2.3. Molecular identification of fungi

2.3.1. DNA extraction

Strains were cultured on yeast extract sucrose (YES) agar for 5 days at 25 °C. Mycelia were scraped from the medium and DNA was extracted using the Easy-DNA kit (Invitrogen, Carlsbad, CA, USA) according to manufacturer instructions.

Fig. 1. Geographical location of the regions where the wheat grain samples were collected. Highlighted areas represent freshly harvest grains collected during the 2012 harvest in Nova Itacolomi (Parana State), Passo Fundo (Rio Grande do Sul State), and Capao Bonito (Sao Paulo State).

2.3.2. Fungal identification

Partial sequencing of the internal transcribed spacer region (ITS) using the ITS1/ITS4 primers (White, Bruns, Lee, & Taylor, 1990) was used for genus identification. *Fusarium* isolates were further identified to species level by sequencing the elongation factor (EF-1α) gene using the EF-1/EF-2 primers (O'Donnell & Cigelnik, 1997). All amplification reactions were carried out in a volume of 25 µl with 1× PCR buffer containing 0.3 mM of each primer, 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.04 U/µl Taq DNA polymerase (Invitrogen, Carlsbad, CA, USA), and 100 ng template DNA. The thermal cycler conditions for PCR were: 1 cycle of 5 min at 94 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 56 °C, 1 min at 72 °C, and 1 cycle of 7 min at 72 °C. After DNA purification (Illustra ExoProStar, GE), sequencing was performed in an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) using the BigDye Terminator v3.1. kit (Applied Biosystems) according to manufacturer instructions. The sequences were edited with the BioEdit v7.0.9.0 software and aligned to sequences of the GenBank and Fusarium ID v1.0 databases. *Microdochium* isolates were identified by conventional PCR following the procedures of Nicholson, Less, Maurin, Parry, and Rezanoor (1996).

2.4. Mycotoxin analysis

2.4.1. Materials and reagents

Mycotoxins standards (zearalenone, zearalanone, deoxynivalenol and deepoxydeoxynivalenol) were purchased from Sigma–Aldrich (Sao Paulo, Brazil). Acetonitrile, methanol and ammonium acetate were purchased from J. T. Baker (Sao Paulo, Brazil). Ultra-pure water was obtained from a Milli-Q-System from Merck Millipore (Bedford, MA, USA).

2.4.2. Extraction of deoxynivalenol and zearalenone

For each sample, three grams of wheat grains were ground and homogenized in 24 ml of methanol/water solution (70:30, v/v) and shaken for 20 min. After shaking, the mixture was filtered through a filter Whatman n° 4, 18 cm. Prior to the liquid chromatography–mass spectroscopy (LC–MS/MS) analysis a 40 µl aliquot was transferred to a vial, mixed with 955 µl of methanol/water solution (50:50, v/v) and added 5 µl of internal standards previously diluted in methanol/water solution (50:50, v/v).

2.4.3. Chromatographic conditions

DON and ZEA were determined by the HPLC Agilent 1200 (Agilent Technologies, Santa Clara, CA, USA) equipped with an API5000 triple quadruple mass spectrometer with an electrospray source (AB Sciex, Concord, ON, Canada). The LC column was a Zorbax-XDB, C8, 200 × 4.6 mm, 3 µm (Agilent Technologies, Santa Clara, CA, USA) with a C8 pre-column cartridge. For the isocratic chromatography procedure, methanol/water (60:40, v/v) containing 0.05 M of ammonium acetate was used as mobile phase, run under a flow rate of 1 ml/min, at a column temperature of 35 °C and an injection volume of 5 µl. The MS source dependent parameters were: curtain gas (CUR) 30 psi (240 kPa of max. 99.5% nitrogen), dry gas (GS1) 50 psi (380 kPa of zero grade air), dry gas (GS2) 20 psi (105 kPa of zero grade air), collision-activated dissociation gas (CAD) 12 (arbitrary unit), source temperature 360 °C and ion spray voltage of 5200 V. Detection was performed in negative ion electrospray using a multiple reaction monitoring (MRM) mode (Table 1).