Crop Protection 65 (2014) 232–237

Contents lists available at ScienceDirect

Crop Protection

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

Short communication

Prevalence of fumonisin-producing *Fusarium* species in Brazilian corn grains

Fabrício Eustáquio Lanza [a, *], Laércio Zambolim [a], Rodrigo Veras da Costa [b], Valéria Aparecida Vieira Queiroz [b], Luciano Viana Cota [b], Dagma Dionísia da Silva [b], André Gomes Coelho de Souza [b], José Edson Fontes Figueiredo [c]

[a] *Plant Protection Laboratory, Federal University of Viçosa, Av. P.H. Rolfs, s/n°, 36570-000, Viçosa, MG, Brazil*
[b] *Plant Pathology Laboratory, Embrapa National Maize and Sorghum Research Center, Rod MG 424, Km 65, Cx. Postal 151, CEP 35701-970, Sete Lagoas, MG, Brazil*
[c] *Molecular Biochemistry Laboratory, Embrapa National Maize and Sorghum Research Center, Rod MG 424, Km 65, Cx. Postal 151, CEP 35701-970, Sete Lagoas, MG, Brazil*

ARTICLE INFO

Article history:
Received 19 July 2013
Received in revised form
29 July 2014
Accepted 7 August 2014
Available online 1 September 2014

Keywords:
Fusarium spp.
Mycotoxins
Species identification
Zea mays

ABSTRACT

The aims of this study were to perform morphological and molecular characterization of *Fusarium* species within the *Liseola* section isolated from corn grains from different geographic regions in Brazil, and to evaluate their potential for fumonisins production. The results showed that *Fusarium verticillioides* is the predominant species (99%) associated with corn grains, and other toxigenic species, *Fusarium proliferatum*, was incidental in Brazilian corn. Although there was a high variability in the total fumonisin production among the isolates (0.01–2.39 μg g^{-1}), all fifty isolates analyzed produce fumonisins. The level of total fumonisin production was not correlated with the geographic origin of the isolates.
© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Brazil is the third largest producer of corn in the world with an average production of 77 million tonnes in the crop year 2012/2013 (Agrianual, 2013; CONAB, 2013). However, the average corn yields in Brazil is still low (4.77 tons per hectare), particularly considering the high-yielding hybrids available on the market.

Diseases are a major factor limiting the increase of corn yields in the tropical humid climate in Brazil (Oliveira et al., 2004). Among the most important pathogens of corn in Brazil are different species of the genus *Fusarium* and these are of great concern to technicians and producers (Oliveira et al., 2004; Munkvold and Desjardins, 1997). Among the *Fusarium* species complex (teleomorph *Gibberella fujikuroi*), which comprises more than 50 species, *Fusarium verticillioides*, *Fusarium proliferatum* and *Fusarium subglutinans* are the main species that infect corn kernels (Krska et al., 1997; Leslie et al., 2006). *F. verticillioides* is the most common fumonisin-producing *Fusarium* species infecting corn kernels (Madania et al., 2013; Rahjoo et al., 2008; Orsi et al., 2000; Munkvold and Desjardins, 1997). *Fusarium* spp. causes a variety of diseases in corn such as root rot, stem rot, ear rot, and rot grain (Munkvold, 2003; Munkvold and Desjardins, 1997). In addition to yield damages caused by *Fusarium* spp., they also produce a variety of secondary metabolites highly toxic to humans and other animals (Munkvold and Desjardins, 1997). Among the mycotoxins produced by *Fusarium* spp., fumonisins are considered the most significant problems due to their high incidence, levels of production, and toxicity (Völkel et al., 2011; Munkvold and Desjardins, 1997). Fumonisins are involved in the inhibition of sphingolipids with serious consequences to the cell structure of eukaryotic organisms (Marasas et al., 2004; Wang et al., 1991). The ingestion of fumonisin-contaminated corn can lead to serious diseases in animals, such as horse leukoencephalomalacia (Giannitti et al., 2011; Marasas et al., 1988), pulmonary edema in swine (Harrison et al., 1990), and hepatotoxicity in rats (Gelderblom et al., 1996). Fumonisins are also directly related to human esophageal cancer and defects in neural tube formation in fetuses (Doi and Uetsuka, 2011; Völkel et al., 2011; Jackson and Jablonski, 2004; Ueno, 2000).

The identification of *Fusarium* species is important for epidemiological purposes and may become absolutely necessary because

* Corresponding author. Fax: +553138991095.
E-mail addresses: fabricio.lanza@ufv.br, falanza@bol.com.br (F.E. Lanza).

http://dx.doi.org/10.1016/j.cropro.2014.08.003
0261-2194/© 2014 Elsevier Ltd. All rights reserved.

corn kernels infected by *Fusarium* are often asymptomatic and the levels mycotoxin may vary from very low to very high, depending upon the contaminant species (Munkvold, 2003). Therefore, the prompt detection and correct identification of *Fusarium* species in corn grains is important to monitor and prevent ear rot disease and the input of mycotoxins in the food chain (Jackson and Jablonski, 2004). The morphological identification of *Fusarium* species may not be sufficient for reliable identification of species (Madania et al., 2013; Rahjoo et al., 2008). DNA sequence-based molecular tools are increasingly used to enable accurate *Fusarium* species determination (Balajee et al., 2009; Wang et al., 2011). For example, AFLP, TEF-1α partial gene sequencing and PCR based on MAT alleles and real time RT-PCR technique based on FUM1 and FUM19 genes were used for identifying *Fusarium* species and to estimate production levels of fumonisins B1 and B2 in one Brazilian corn hybrid planted in four localities (Rocha et al., 2011). However, these approaches are costly, time-consuming and not readily available in all laboratories (Wang et al., 2011). Thus, the analysis of rRNA genes and the use of species-specific primers for species identification are more frequently used (Covarelli et al., 2012; Mulè et al., 2004; Rahjoo et al., 2008).

In the present study, morphological characters and PCR with species-specific primers were used for rapid identification of *Fusarium* species from section *Liseola* associated with corn grains isolated from thirteen Brazilian hybrids planted in the fifteen major maize production regions, and a low cost immunoaffinity chromatography was used to characterize the potential for total fumonisin production of these *Fusarium* spp.

2. Materials and methods

This study was performed in the Plant Pathology laboratory at the National Maize and Sorghum Research Center – CNPMS – EMBRAPA, Sete Lagoas – MG, Brazil. The identification of the *Fusarium* species consisted of morphological characterization and molecular confirmation of species.

Two corn cobs with both asymptomatic and symptomatic kernels (radial streaks, centered on the stylar canal or kernel rot, typical of *Fusarium* infection) were collected from each of the 15 major corn producing regions in Brazil. These regions are characterized by a great diversity of climatic conditions (Fig. 1).

In the laboratory, the cobs were thrashed; homogenized and 200 grains per sample were randomly selected to perform the Blotter test. The grains were surface desinfested by immersion in 2% sodium hypochlorite for five minutes and then washed twice with sterile distilled water. Afterward, each grain sample was equally distributed in eight germination boxes containing a filter paper moistened with 5% agar-water (w/v) according to Pinto et al. (2007). The germination boxes were maintained at room temperature under continuous light for 24 h to stimulate germination, and then transferred to a freezer at −5 °C for a period of 24 h. Thereafter, the germination boxes were incubated at 25 °C with 12 h photoperiod for 10 days. The morphological identification, based on colony morphology (cottony white to pinkish mycelium and presence of microconidia) and quantification of the pathogens growing on grains were made with the aid of a stereoscopic microscope and a binocular microscope. Grains showing characteristic colonies of *Fusarium* spp. were transferred to Petri dishes containing PDA medium (potato dextrose agar) to obtain monoconidial cultures. We obtained 230 single spore isolates of *Fusarium*, and each monosporic culture was categorized based on the date of collection, corn genotype and geographic location of the sample. Pre-cultured isolates in PDA medium were preserved by the addition of mineral oil on the top of the culture and stored at 25 °C in the dark.

For morphological identification, all isolates were grown on PDA and incubated at 25 °C with 12 h photoperiod for 14 days. The macroscopic characteristics of the colony were analyzed with the aid of a dissecting and a compound microscope. The morphology of microconidia, macroconidia, conidiogenous cells (phialides) and chlamydospores of each species were identified according to the criteria proposed by Leslie et al. (2006).

For molecular identification, the isolates were cultured in PD (potato dextrose) broth for 3 days on an orbital shaker at 90 rpm under continuous fluorescent light. After this period the mycelium was collected with the aid of sterile gauze and frozen in liquid nitrogen. Total DNA was extracted using the protocol described by Murray and Thompson (1980). The species-specific primer pairs VER1/VER2, PRO1/PRO2 and SUB1/SUB2, designed by Mulè et al. (2004), were used for *F. verticillioides*, *F. proliferatum*, and *F. subglutinans* identification, respectively. PCR reactions were performed separately for each set of primers containing 200 mM Tris–HCl (pH 8.4), 500 mM KCl, 1.5 mM $MgCl_2$, 0.5 μM each primer,

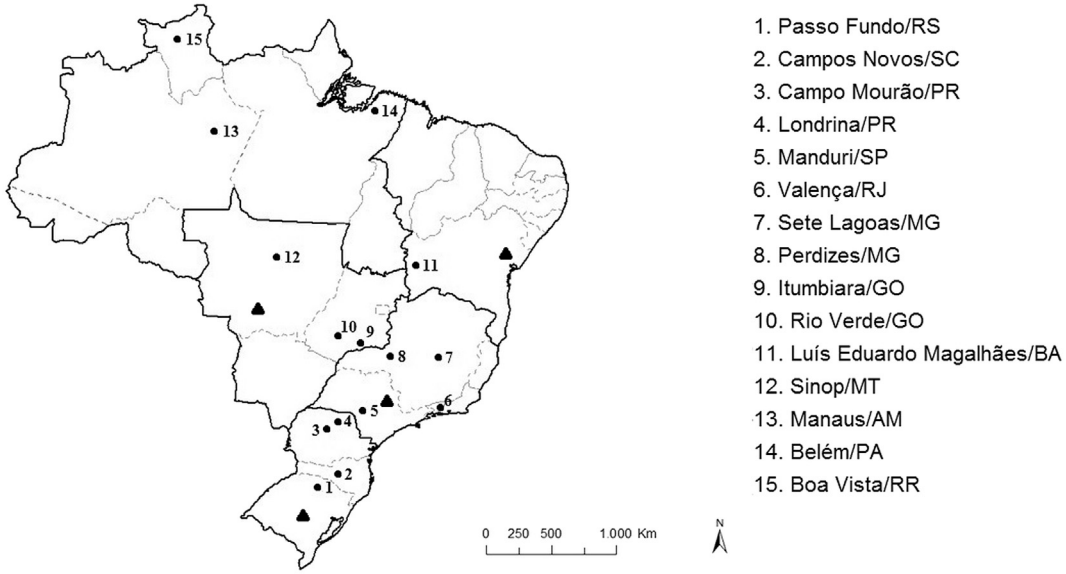

Fig. 1. Brazilian corn planting areas sampled for *Fusarium* species in the present study (●) and Rocha et al. (2011) (▲).

Download English Version:

https://daneshyari.com/en/article/4505830

Download Persian Version:

https://daneshyari.com/article/4505830

Daneshyari.com