Crop Protection 98 (2017) 70–75

Contents lists available at ScienceDirect

Crop Protection

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

Short communication

Fumonisin B1-producing *Fusarium* species from agricultural crops in Malaysia

Nor Azliza Ismail, Masratul Hawa Mohd, Nik Mohd Izham Mohamed Nor, Latiffah Zakaria*

School of Biological Sciences, Universiti Sains Malaysia, 11800 USM Penang, Malaysia

ARTICLE INFO

Article history:
Received 3 November 2016
Received in revised form
13 March 2017
Accepted 14 March 2017

Keywords:
Fusarium spp.
Fumonisin B1
Agricultural crops

ABSTRACT

Many species of *Fusarium* are pathogenic as well toxigenic to a wide variety of plants. The present study was conducted to determine the ability of four members of *Fusarium fujikuroi* species complex and *F. oxysporum* from various crops to produce fumonisin B1 (FB_1). Isolates of *Fusarium* species from infected parts of asparagus, ginger, oil palm, mango, banana, maize, and rice were identified as *F. verticillioides* (11 isolates), *F. proliferatum* (50 isolates), *F. fujikuroi* (24 isolates), *F. andiyazi* (six isolates), and *F. oxysporum* (32 isolates). *FUM1*, a gene involved in fumonisin biosynthesis, was detected in 94 isolates of *F. verticillioides* (11 isolates), *F. proliferatum* (49 isolates), *F. fujikuroi* (24 isolates), and *F. oxysporum* (10 isolates) but only 61 were positive for FB_1 when tested using RIDA®Quick Fumonisin test strip, indicating that the presence of *FUM1* was not necessarily associated with FB_1 production. Based on ultra-high performance liquid chromatography, all the 61 isolates were detected to produce FB_1, at variable levels, with concentrations ranging from 0.60 to 29.2 μg/g. Our results suggested that there is a potential risk of FB_1 contamination in agricultural crops in Malaysia.

1. Introduction

Toxigenic *Fusarium* species can produce mycotoxins under favorable environmental conditions. Fumonisin B1 (FB_1) is one of most common mycotoxin produced by toxigenic *Fusarium*, worldwide. This mycotoxin is produced by several members of *Fusarium fujikuroi* species complex (FFSC), including *F. verticillioides*, *F. proliferatum*, *F. nygamai*, and *F. fujikuroi* as well as by *F. oxysporum*, which is not a member of FFSC (Chandra Nayaka et al., 2010; Cruz et al., 2013; Nelson et al., 1992; Waśkiewicz et al., 2009). Fumonisin B_1-producing *Fusarium* species can infect a wide variety of crops and can, therefore, contaminate a variety of food and feed. Natural occurrence of FB_1 has been reported in many agricultural crops, including maize, rice, wheat, barley, oat, hops, sorghum, millet, soybean, and asparagus (Abbas et al., 1998; Liu et al., 2005; Shetty, 2011).

Various methods have been developed to investigate the production of FB_1 by *Fusarium* species infecting agricultural crops. One of the methods, developed by Proctor et al. (1999), involves the detection of *FUM* gene which is an essential gene in fumonisin biosynthesis. The detection of this gene is useful and allows rapid and accurate prediction of FB_1 production by *Fusarium* species because it is able to distinguish fumonisin-producing and non-producing strains (Stępień et al., 2011a). A semi-quantitative method using immunochromatographic test strip has also been developed, which can provide convenient and rapid method for detection of FB_1; this test strip is commercially available.

Quantification of FB_1 using Ultra-high Performance Liquid Chromatography- Fluorescence detection (UHPLC-FLD) can shorten the time of analysis because of smaller particle size of the column and highly porous which allows faster flow rate (Khayoon et al., 2010). UHPLC might also provide efficient quantification and separation of FB_1 because of high speed analysis, greater resolution, and high sensitivity (Soleimany et al., 2012).

Fusarium species are common field fungi that can infect various types of agricultural crops. Therefore, it is important to detect the fungus and to determine the potential of different *Fusarium* species associated with various agricultural crops to produce FB_1. The production of FB_1 may also indicate the mycotoxicological risk to humans and animals that consume the produce. Thus, the present study was performed to determine the potential of five *Fusarium* species, *F. verticillioides*, *F. andiyazi*, *F. proliferatum*, *F. fujikuroi*, and *F. oxysporum* from various crops to produce FB_1. The methods employed in the study were detection of *FUM1* gene using

* Corresponding author.
E-mail addresses: Lfah@usm.my, latiffahz@yahoo.com (L. Zakaria).

http://dx.doi.org/10.1016/j.cropro.2017.03.014
0261-2194/

polymerase chain reaction (PCR) and immunochromatographic test strip, and quantification using UHPLC-FLD.

2. Materials and methods

2.1. Fusarium isolates

A total of 123 isolates of *Fusarium* species, which are potentially FB_1 producers, were selected from Plant Pathology Laboratory culture collection, School of Biological Sciences, Universiti Sains Malaysia, Penang, Malaysia. These were isolated from the infected parts of asparagus, ginger, oil palm, mango, banana, maize, and rice from various locations in 13 Malaysian states (Supplementary Table). The 123 isolates were identified to belong to five species, namely *F. proliferatum* (50 isolates), *F. fujikuroi* (24 isolates), *F. verticillioides* (11 isolates), *F. andiyazi* (six isolates), and *F. oxysporum* (32 isolates), using translation elongation factor 1α gene (TEF-1α) sequences. The gene was amplified using EF1 (5′-ATG GGT AAG GAG GAC AAG AC-3′) and EF2 (5′-GGA AGT ACC AGT GAT CAT GTT-3′) primer pair (O'Donnell et al., 1998). After sequencing, the consensus sequence was compared with the existing sequences present in Fusarium-ID database (http://isolate.fusariumdb.org/index.php) using BLAST.

2.2. Detection of FUM1

For amplification of *FUM1* gene, *FUM1* specific primers, Fm1 (5′-GACACCTCC TTCTTCTCCATG-3′) and Fm2 (5′-GTGCCGGTTCCGTGTGCTTC-3′), were used (Proctor et al., 1999). The number of cycles and conditions of PCR were based on the method described by Dissanayake et al. (2009). PCR was performed in 20 μL reaction mixture consisting of 2.5 μL 10× PCR buffer, 1 μL $MgCl_2$ (2.5 mM), 1.25 μL of both primers (20 mM each primer), 0.5 μL *Taq* DNA polymerase (5 U; Promega, Madison, WI, USA), 1 μL dNTP mix (200 mM) (Promega), 1 μL of DNA template (~100 ng), and sterile distilled water to make the volume to 20 μL. For positive controls, two reference strains each of *F. verticillioides* (A149 and A999) and *F. proliferatum* (D4583 and D4584) obtained from Kansas State University, USA were included. Sterile distilled water was used as a negative control.

The PCR amplification was performed in a Bio-Rad Thermal Cycler (MyCycler™, Bio-Rad, California, USA) with the following conditions: an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 15 s, annealing at 65 °C for 30 s, extension at 72 °C for 1.5 min, a final extension at 72 °C for 5 min, and final hold at 4 °C.

The PCR products were detected by 1% agarose gel electrophoresis in 1× Tris Borate-EDTA buffer, performed at 90 V and 400 mA for 90 min. The size of the PCR products was estimated by comparison with 1 kb DNA ladder (Thermo Scientific GeneRuler: Waltham, VA, USA). The gels were stained with ethidium bromide for 30 min, visualized under UV light, and the image was captured using Molecular Imager® Gel Doc™ XR Systems (Bio-Rad). The gel images were then quantified using The Discovery Series™ Quantity One® 1-D Analysis software.

2.3. In vitro production of FB_1

About 25 g of cracked maize, adjusted to 45% moisture content, were filled into 150-mL Erlenmeyer flasks and autoclaved at 121 °C for 1 h. After three days, the cracked maize was inoculated with 1 mL conidial suspensions (~1×10^7 conidia/mL) from 7-day-old cultures of *Fusarium* isolates grown on potato dextrose agar (PDA) medium. The inoculated cracked maize cultures were incubated at 25 °C for 14 days (Falcao et al., 2011). The control was treated in the same manner except that the cracked maize was inoculated with sterile distilled water.

2.4. Detection of FB_1 using test strip

Fusarium isolates, in which the presence of *FUM1* was detected, were used in this test. The FB1 production was detected using RIDA®Quick Fumonisin test strip RQS (R-Biopharm, Darmstadt, Germany) following the manufacturers' instructions. Two gram of *Fusarium*-inoculated maize was mixed with 25 μL extraction buffer. The mixture was evenly agitated and incubated for 5 min. About 200 μL of the mixture was applied on the test strip. The stop solution was added after the incubation period. The samples were scored as positive when the test line was visible and negative when no test line was visible. The detection limit was 0.3 μg/g.

2.5. FB_1 analysis using UHPLC-FLD

The *Fusarium* isolates that produced FB_1 using RIDA®Quick Fumonisin test strip RQS were used in UHPLC-FLD analysis. FB_1 extraction was performed according to the method described by Holcomb and Thompson (1996). A total of 10 g of the inoculated cracked maize was initially extracted with 40 mL of acetonitrile (ACN): water (50:50, v/v). The extracts were filtered through Whatman no. 4 filter paper. The raw filtrate was loaded onto a C18-SPE tube (Supelco, PA, USA), which was preconditioned with 5 mL methanol and 5 mL 1% KCl solution. About 2.5 mL of 1% KCl was passed through the tube after loading of the sample. The tube was then washed with 3 mL of 1% KCl solution and 2 mL of ACN:1% KCl mixture (10:90, v/v). The residue containing FB_1 was then eluted off with 2 mL of ACN:water (50:50, v/v). Derivatisation of FB_1 was performed by adding 800 μL n-Octylphosphonic acid (OPA) reagent into 200 μL samples prior to the chromatographic analysis. FB_1 was quantified using an Acquity UHPLC™ system (Waters, Milford, MA, USA) equipped with BEH C18 stainless steel column (2.1 × 100 mm), with 1.7-μm particle size, connected to FLD (Waters). The excitation and emission wavelength were set at 335 and 440 nm, respectively. The mobile phase consisted of methanol and 0.1 M sodium dihydrogen phosphate (NaH_2PO_4) (78:22 v/v; adjusted to pH 3.35 with o-phosphoric acid), filtered through 0.22 μm nylon transfer membrane filter. The flow rate was at 0.1 mL/min. Purified FB_1 (Sigma Aldrich, WI, USA) was used as a standard for FB_1 quantification.

3. Results

3.1. Detection of FUM1

The *FUM1* amplicons of the expected size (780 bp) were amplified from 94 isolates. *FUM1* was detected in 11 isolates of *F. verticilliodes*, 49 isolates of *F. proliferatum*, 24 isolates of *F. fujikuroi*, and 10 isolates of *F. oxysporum* (Table 1). The gene was not detected in six isolates of *F. andiyazi*, one isolate of *F. proliferatum*, and 22 isolates of *F. oxysporum.*

3.2. Detection of FB_1 using test strip

The rapid detection of FB_1 using RIDA®Quick Fumonisin test strip showed that 61 isolates were positive for FB_1 production. The color intensity of the test lines and the presence of FB_1 were observed in nine isolates of *F. verticillioides*, 39 isolates of *F. proliferatum*, three isolates of *F. fujikuroi*, and 10 isolates of *F. oxysporum.* Thirty-four isolates comprising two isolates of *F. verticillioides*, 11 isolates of *F. proliferatum*, and 21 isolates of *F. fujikuroi* did not produce FB_1 although the presence of *FUM1* was detected (Table 1).