تعريف مورفولوجي وجزيئي للفطر *Fusarium culmorum* والكشف عن العزلات المنتجة للتريكوثيسينات من القمح المصاب في سورية

Morphological and Molecular Identification of *Fusarium culmorum* and Detection of Trichothecene-Producing Isolates from Infected Wheat in Syria

ليلى زيدان[1] أغيد صالح[2] دانا جودت[2] وليد نفاع[3]

Laila Zidan[1] Aghyad Saleh[2] Dana Jawdat[2] Walid Naffaa[3]

walid1851966@yahoo.com or ray-dya@scs-net.org

(1) جامعة دمشق، كلية الهندسة الزراعية الثانية، السويداء، سورية

(1) Faculty of Agriculture, Damascus University, Sweida branch, Syria.

(2) قسم البيولوجيا الجزيئية والتقانات الحيوية، هيئة الطاقة الذرية، دمشق، سورية.

(2) Atomic Energy Commission, Damascus, Syria.

(3) جامعة دمشق، كلية الهندسة الزراعية، قسم وقاية النبات، دمشق، سورية.

(3) Department of Plant Protection, Faculty of Agriculture, Damascus University, Damascus, Syria.

الملخص

يُعد النوع Fusarium culmorum أحد المسببات المرضية المهمة على القمح، والمعروف بإنتاجه للسموم الفطرية التي تشكل خطراً على صحة الإنسان والحيوان. أجريت هذه الدراسة لتعريف وتوصيف 14 عزلة سورية من النوع *F. culmorum*، والكشف عن العزلات المنتجة للتريكوتيسينات Trichothecenes. تم الحصول على العزلات من جذور وتاج نباتات قمح تبدي أعراض إصابة أنموذجية، جُمعت من أربع محافظات سورية. صُنّفت العزلات المتحصل عليها بالطرائق المورفولوجية والجزيئية. أظهرت النتائج أن المسبب الأكثر أهميةً لعفن الجذور والتاج على القمح في المناطق المدروسة كان النوع *F. culmorum*، والذي تم تعريفه بالاعتماد على الصفات المورفولوجية، وبالطرائق الجزيئية باستخدام بادئات متخصصة بهذا النوع، والتسلسل النيكليوتيدي للمورثة TEF1-α. أجري تفاعل البلمرة المتسلسل PCR باستخدام زوج من البادئات المتخصص بالمورثة Tri-5 للكشف عن قدرة العزلات على إنتاج التريكوتيسينات. أظهرت النتائج أن جميع العزلات تحتوي على المورثة Tri-5، إذ تم تضخيم قطعة من الدنا بحجم 544bp. يُعد تحديد أنواع الفيوزاريوم التي تصيب القمح في سورية، وقدرتها على إنتاج السموم الفطرية، أمراً ضرورياً جداً لتقدير خطر التلوث بالسموم الفطرية، وتطوير استراتيجيات للحد من الإصابة بأمراض الفيوزاريوم، وتلوث الأغذية والأعلاف بالسموم التي تنتجها.

الكلمات المفتاحيّة: *F. culmorum*، قمح، تريكوتيسينات، بادئات متخصصة، TEF1-α.

ISSN:2305 - 5243 ; AIF-181 (p:1 - 9 E)

Abstract

Fusarium culmorum is an important pathogen of wheat and known to produce mycotoxins which are potential health hazard for both humans and animals. This study was carried out to identify and characterize 14 Syrian isolates of F. culmorum and to detect trichothecene-producing isolates. The isolates were recovered from crowns and roots of wheat plants, showing typical symptoms, collected from four Syrian provinces. Each isolate was taxonomically identified using both morphological and molecular techniques. The results showed that the most important causal agent of root and crown rot on wheat in the investigated region was identified as *F. culmorum* based on morphological characteristics, amplification of specific primers and TEF1-α sequences. All fourteen isolates were analyzed by PCR for trichothecene producing ability using Tri-5 gene-based primers. The results showed that all isolates had a Tri-5 gene and amplified the expected DNA fragment of 544 bp. Identification of the *Fusarium* species that infect wheat in Syria and, what is more important, their ability to produce mycotoxins, is needed to estimate the risk of mycotoxin contamination and to develop strategies to reduce the incidence of *Fusarium* diseases and mycotoxin contamination of food and feed.

Keywords: *F. culmorum*, Wheat, Trichothecene, Specific primers, TEF-1α.

Introduction

Fusarium culmorum (W.G. Smith) Saccardo is a soil-borne fungus able to cause root rot, crown rot and Fusarium head blight on different small-grain cereals, in particular wheat and barley (Scherm *et al*., 2013) .

F. culmorum belongs to section Discolour, along with *F. graminearum*, *F. sambucinum*, *F. crookwellense*, *F. trichothecioides* and *F. heterosporum* (Joffe, 1974; Nelson *et al*., 1983; Ismail *et al*., 2015). It causes severe damage on yield quality and quantity and results in contamination of the grain with mycotoxins (Goswami and Kistler, 2004; Matny, 2015).

The major mycotoxins produced by *F. culmorum* are trichothecenes, zearalenone, moniliformin and fusarins (Leslie and Summerell, 2006; Demeke *et al*., 2005). These mycotoxins threat humans and animals consuming contaminated food or feed (Leslie and Summerell, 2006; Matny, 2015), so detection and control of Fusarium species is crucial to prevent toxins entering the food chain (Jurado *et al*., 2006).

Many methods have been used for *Fusarium* identification including morphological and molecular methods. The morphological criteria that are useful for identification of *Fusarium* species include: primary characters which include shape of the macroconidia, presence or absence of microconidia, their shape and mode of their formation, nature of the conidiogenous cells bearing microconidia, and secondary characters such as colony morphology, growth rate and pigmentation (Burgess *et al*., 1994; Leslie and Summerell, 2006).

Polymerase chain reaction (PCR) can be also used for the identification and confirmation of Fusarium species and for detection of mycotoxin-synthesis-pathway genes to determine the ability of the isolates to produce mycotoxins.

Several diagnostic PCR assays have been developed for F. culmorum. Schilling *et al*. (1996) and Nicholson et al. (1998) designed specific primer. In addition, for Fusarium species identification, sequence determinations of the translation elongation factor 1-alpha (*TEF1-α*) gene have commonly been used and sequences for this gene are available through GenBank and through the FUSARIUM-ID databases (Geiser *et al*., 2004).

The trichothecene pathway is well explored, and several trichothecene biosynthetic genes have been characterized (Desjardins *et al*., 1993). Many researchers targeted the gene TRI5 (Doohan *et al*., 1999) and the gene TRI6 (Bluhm *et al*., 2002, 2004) which are involved in trichothecene biosynthesis to detect trichothecene-producing *Fusarium* spp.

The main objectives of this study are the identification of *F. culmorum* based on morphological characteristics followed by molecular analysis confirmation and detection of the presence of *TRI5* gene producing isolates by using specific primers.

Materials and Methods

-Sample collection: Infected wheat plants were collected from different regions of four Syrian provinces [Tartous, Latakia, Hama (Al-Ghab plain), and Sweida], during 2017 and 2018. Each sample was stored in a paper envelope. In the laboratory, roots and crowns of diseased samples were washed under running tap water and cut into small pieces (~ 3cm) for fungal isolation.

-Isolation of F. culmorum: Pieces of infected roots and crowns were surface-sterilized with 1% sodium hypochlorite for 3 min, rinsed in sterile distilled water and dried in a laminar flow cabinet, then placed separately on potato dextrose agar (PDA) with 0.3 g L^{-1} streptomycin and neomycin sulphate and incubated at 22±1°C in the dark for 7 days. Isolates of *Fusarium* species from infected parts of the roots were purified. A total of 14 *Fusarium* isolates were recovered from wheat samples collected from four Syrian provinces.

-Morphological identification of *F. culmorum*: The isolates were identified based on the morphological characteristics of single-spore isolates as described by (Nelson *et al*., 1983; Burgess *et al*., 1994). Single-spore cultures were grown on PDA in the dark at 25°C and 30°C for 72 h to study the growth rate as described by Burgess *et al*. (1994). Two media were used for the identification study: Potato Dextrose Agar (PDA) to study the growth rate and cultural appearances (colony colour and pigmentations); and Carnation Leaf Agar (CLA) to investigate microscopic characteristics as described by Burgess *et al.* (1994) and Leslie and Summerell (2006). The morphological characters of the isolates were compared with the original morphological species description as outlined in Burgess *et al*. (1994), and Leslie and Summerell (2006).

Molecular identification of *F. culmorum*:

-DNA extraction: Fungal isolates were grown on PDA medium and incubated for 3–7 days at 25°C. The mycelium was harvested, transferred to a micro-centrifuge tube and suspended in 400µl extraction buffer (1.4 M sodium chloride, 0.1M Tris HCl, pH = 8, 20 mM EDTA Ethylenediaminetetraacetic acid, 2% CTAB Cetyl rimethyl ammonium bromide, 1% PVP polyvinyl pyrrolidon, 1% β-mercaptoethanol). Lysis of the mycelium was achieved by the addition of acid washed 0.4-0.6 mm diameter glass beadsand 400µl phenol/chloroform/iso-amyl alcohol (Phe/Chl/IAA) (25:24:1). The mixture was vortexed for 10 min, centrifuged at 14000 rpm for 5 min, and 500 µl of supernatant were transferred to a new micro-centrifuge tube. 1000 µl of ice-cold ethanol 100% were added, mixed gently, incubated at -20°C for 1 h and centrifuged at 12000 rpm for 10 min to pellet the DNA. Supernatant was decanted, and DNA pellet was washed with 1000 µl of 70% ethanol. DNA pellet was air dried and dissolved in 50-75 µl TE buffer (10 mMTris-HCl pH 8.0, 0.1 mM EDTA). 2µl RNase was added to DNA samples, mixed and incubated at 37 °C for 45 min (Zidan *et al*., In press).

The DNA concentration was measured using NanoDrop Spectrophotometers (Thermo Scientific), and was adjusted to 100 ng/µL. DNA was then used as template for subsequent amplification using PCR.

-Species-specific PCR: Species-specific PCR amplifications were carried out using primers Fc01F (5'-ATGGTGAACTCGTCGTGGC -3') and Fc01R (5'-CCCTTCTTACGCCAATCTCG-3') for the detection of *F. culmorum* (Nicholson *et al*., 1998). The PCR reaction, in a total volume of 25 µl, included 12.5 µl *Dream Taq Green* PCR Master Mix (2X) (Thermo Scientific), 7.5 µl RNase-free water, 1.5 µl of each primers (10 µM) and 2 µl of DNA. The following PCR program conditions were applied: 95 °C for 5 min, 5 cycles (95°C for 30 sec, 66°C for 30 sec, 72°C for 30 sec), 5 cycles (95°C for 30 sec, 64°C for 30 sec, 72°C for 30 sec), 25 cycles (95°C for 30 sec, 62°C for 30 sec, 72°C for 30 sec), and final extension at 72 °C for 8 min.

Amplified products were analyzed using 1% agarose gel electrophoresis, and visualized under a UV transilluminator.

Identification of *F. culmorum* using partial translation elongation factor 1-α (TEF1-α) sequence: Partial *TEF-1α* gene sequence was amplified using primers EF1 (ATGGGTAAGGAGGACAAGAC) and EF2 (GGAAGTACCAGTGATC ATGTT) (O'Donnell *et al*.,1998). The TEF partial sequence region was amplified in a 25 µl reaction mixture containing 12.5 µl *DreamTaq Green* PCR Master Mix (2X)(Thermo Scientific), 7.5 µl

RNase-free water, 1.5 µl of each primer (10 µM)and 2 µl of DNA. The PCR conditions were as follows: pre-denaturation at 95 °C for 3 min; 10 cycles of denaturation at 94 °C for 1 min, annealing at 53 °C for 1 min, and extension at 72 °C for 2 min and final extension at 72 °C for 10 min.

Amplified products were analyzed using 1% agarose gel electrophoresis, and visualized under a UV transilluminator. Amplified samples showing discrete bands were subjected to automated DNA sequencing.

-Detection of the potential of *F. culmorum* isolates to produce trichothecene using specific primers.

Species-specific PCR amplifications were carried out using primers Tri5F (5'-AGCGACTACAGGCTTCCCTC-3') and Tri5R (5'- AAACCATCCAGT TCTC CATCTG-3') for the detection of Tri-5 gene producing isolates which encodes the key enzyme in trichothecene production (Doohan et al., 1999). The PCR reaction, in a total volume of 25 µl, included 12.5 µl *Dream Taq Green* PCR Master Mix (2X) (Thermo Scientific), 7.5 µl RNase-free water, 1.5 µl of each primer (10 µM) and 2 µl of DNA. The PCR conditions were as follows: pre-denaturation at 95 °C for 3 min; 30 cycles of denaturation at 95 °C for 30 sec, annealing at 62 °C for 20 sec, and extension at 72 °C for 45 sec and final extension at 72 °C for 10 min.

Amplified products were analyzed using1% agarose gel electrophoresis, and visualized under a UV transilluminator

Results

-Morphological characterization: A total of fourteen isolates of *Fusarium* (FTZ14, FTZ16, FTZ18, FTZ27, FTZ43) from Tartous, (FLZ20) from Latakia, (FGZ9, FGZ10, FGZ21, FGZ29, FGZ40) from Hama, (FSZ59, FSZ61, FSZ70) from Sweida, were recovered from infected roots and crowns of wheat plants. They were identified as *F. culmorum* based on morphological characteristics .

On potato dextrose agar, growth was rapid at the optimum temperature of 25°C, the colony diameter ranged from 5.8 - 6.1cm at 25°C and 1.8- 2.4 cm at 30°C after incubation in the dark for 3 days.

Some isolates produced dense aerial mycelium, white, rose or light yellow (Fig.1A, B, C), and others produced yellow mycelium around the central spore mass and white at the apex (Fig.1D). Abundant yellow or orange sporodochia developed in some isolates as the culture ages (Fig.2). These isolates formed rose and yellow or rose to burgundy pigments in the agar (Fig.1E, F, G, H).

Macroconidia were produced in sporodochia from branched monophialides on carnation leaf agar (Fig.3D). They were short and stout, curved, usually 3–5 septate (Fig.3A), with blunt (rounded) apical cells (Fig.3B), or slightly papillate (Fig.3C), the basal cells were blunt or notched. Microconidia were absent. Chlamydospores were formed, intercalary in the hyphae, solitary, in pairs, in chains or in clumps (Fig.3E).

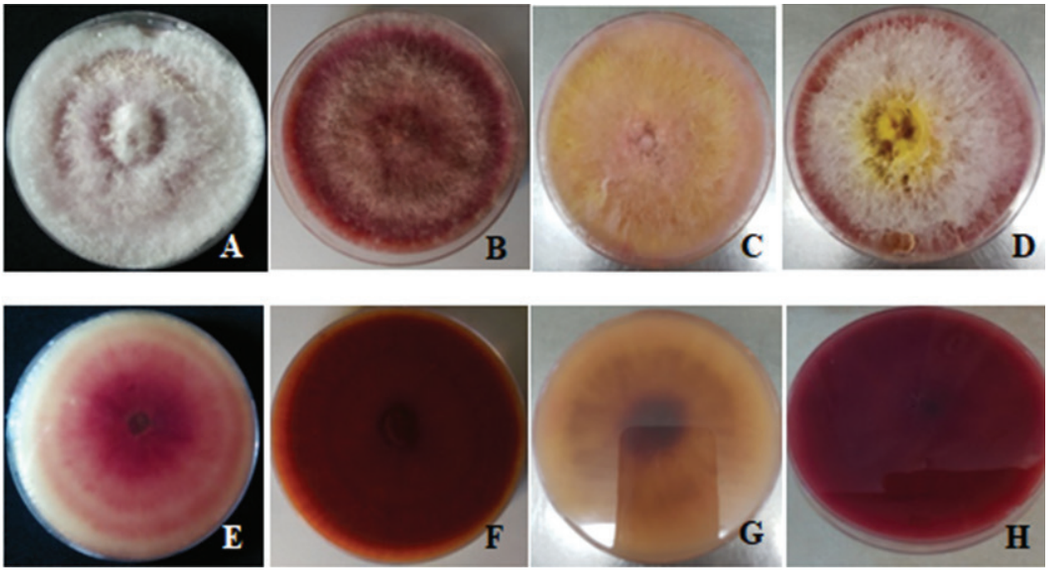

Figure 1. Colonies of some *F.culmorum* isolates on PDA medium. (A, B, C, D): upper surfaces, (E, F, G, H): lower surfaces.

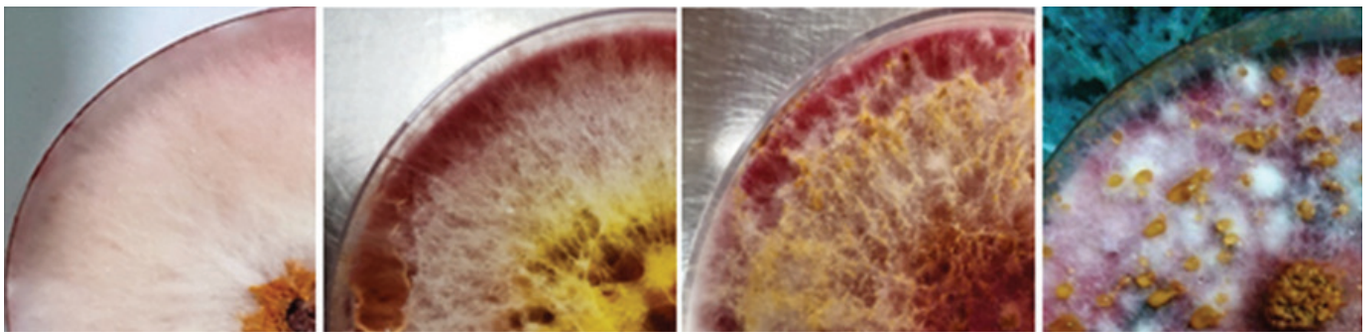

Figure 2. Distribution of sporodochia on PDA in some isolates.

Figure 3. Morphological characteristics of *F.culmorum*: (A) macroconidia, (B) macroconidia with a blunt apical cell, (C) macroconidia with a papillate apical cell, (D) branched monophialides, (E) chlamydospores.

Molecular identification of *F. culmorum*

-Detection of *F. culmorum* using specific primers: Identification of the fourteen isolates was conducted by using specific primers Fc01F/R. The PCR has amplified a single fragment with size approximately 570 bp in all isolates except FLZ20 (Fig.4).

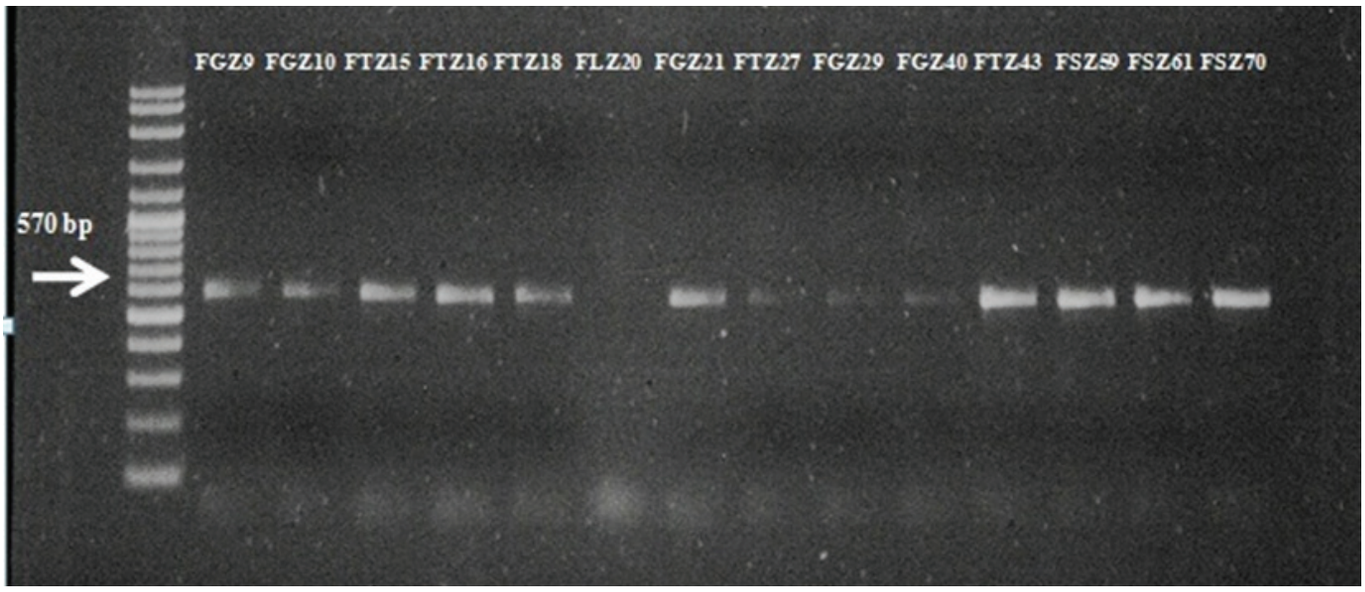

Figure 4. Amplification with species primers Fc01F/R.

-Detection of *F. culmorum* using (*TEF1-α*) gene: DNA from the FLZ20 isolate which showed no positive signal in the species-specific PCR assay was taken for further amplification of the TEF region using EF1 and EF2 primers.

Successful PCR amplification of *TEF-1α*, showing a single band of ~ 700 bp, was obtained in this isolate, and the PCR product was later sequenced for verification.

DNA sequence-based identification of the isolate FLZ20 was achieved. Based on the BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) search of translation elongation factor 1-alpha (*TEF1-α*) sequences, this isolate showed 99,42 similarity with *F. culmorum* . Sequencing result confirmed our morphological identification of FLZ20 as *F. culmorum.*

-Detection of the potential of *F. culmorum* isolates to produce trichothecene using specific primers.

Detection of potential trichothecene producers was positive for all F. culmorum isolates, the amplification yielded the expected band (size 544 bp) (Fig.5).

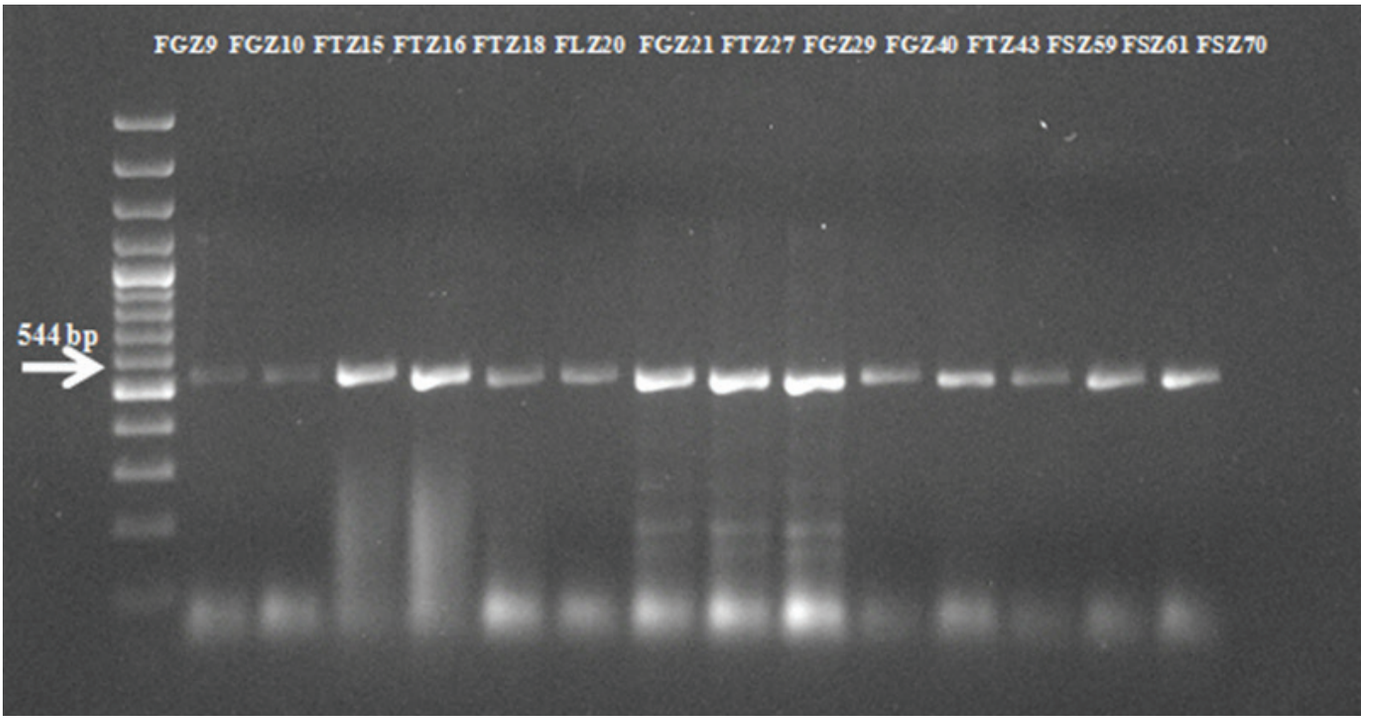

Figure 5. PCR analysis for the trichothecene - producing *Fusarium* isolates genomic DNA using Tri5F/R primers.

Discussion

Several species of *Fusarium* were found in association with wheat crop worldwide. Together with *F. graminearum* and *F. pseudograminearum*, *F. culmorum* have been reported as of the main pathogens of wheat worldwide (Goswami and Kistler, 2004; Wang *et al*., 2006; Wagacha and Muthomi, 2007; Hogg *et al*., 2010).

In our study and based on the descriptions in the Laboratory Manual for Fusarium Research (Burgess *et al*., 1994), and the *Fusarium* Laboratory Manual (Leslie and Summerell, 2006), the fourteen isolates of *Fusarium* recovered from infected crowns and roots of wheat were identified to be as *F. culmorum*.

Fusarium culmorum is most likely to be confused with other species like *F. sambucinum*, as they can be isolated from similar hosts and climatic regions (Burgess *et al*., 1994; Leslie and Summerell, 2006).

The macroconidia of some isolates of *F. culmorum* show some similarity with macroconidia produced by isolates of *F. sambucinum*, the apical cell of *F. culmorum* is usually blunt but in some isolates it is slightly papillate which can lead to confusion with those of *F. sambucinum*. However, the rapid growth rate of *F. culmorum* on potato dextrose agar distinguishes it from *F. sambucinum*, which grows slowly. In addition, the macroconidia

of *F. culmorum* are usually shorter and wider than those of F. sambucinum (Burgess *et al*., 1994; Leslie and Summerell, 2006).

In order to protect human and animal health, the precise identification of the *Fusarium* species is critical to predict the potential mycotoxigenic risk of the isolates, so there is a need for accurate methods which permit a rapid and reliable specific diagnosis of *Fusarium* species.

Polymerase chain reaction (PCR) and real-time PCR tools have been developed for the detection and/or quantification of *F. culmorum* in culture and in naturally infected plant tissue (Scherm *et al*., 2013).

In our study we use two molecular methods to confirm the identification of our isolates after the morphological characterization. Species-specific primers for *F. culmorum*, Fc01F/R were used. Thirteen isolates of the total fourteen were confirmed as F. culmorum, but we could not confirm the morphological identification for FLZ20 isolate. Species-specific primers have been developed and used for PCR detection as well as screening of *Fusarium* spp. (Spanic *et al*., 2010; Abedi-Tizaki and Sabbagh, 2012). Schilling *et al*. (1996) used Species-specific primers (OPT18F/R) for the detection of *F. culmorum*, this pair amplified as expected from template of 65 out of 69 isolates of F. culmorum obtained from various countries and continents. Rahjoo *et al*. (2008) stated that some species-specific PCR primers have been developed, but in most cases they have yet to be more widely tested, and their reliability for analyses of strains from various crops and/or geographic locations is unproven.

Due to the fact that using morphological characteristics and species-specific primers can result in misidentification according to Rahjoo *et al*. (2008), there was a need for another molecular method as an additional criteria to support morphological identification of *Fusarium* spp. The method of choice by several research groups is the characterization of *Fusarium* isolates based on the TEF1-α gene sequences.

Our result based on the partial DNA sequence of the TEF region in the FLZ20 isolate, initially identified as *F. culmorum* by morphological characterization but could not be identified using the Fc01F/R primer pair, showed that it was *F. culmorum*.

Mycotoxins are secondary metabolites produced by fungi which are harmful to both animals and humans. The major mycotoxins produced by *F. culmorum* are trichotecens, zearalenone and fusarins (Summerell and Leslie, 2011).

In our study we targeted the TRI5 gene that encodes trichodiene synthase, which catalyzes the first step in the trichothecene biosynthetic pathway (Doohan *et al*., 1999). Tri5F/ Tri5R primer set was used to determine the potential of the analyzed isolates to produce trichothecenes. The 14 isolates produced the expected DNA fragment, that indicates the presence of the TRI5 gene in these isolates. The development of TRI5 gene specific primers has allowed trichothecene producing *Fusarium* spp. to be distinguished from nonproducing species using PCR-based assays (Niessen and Vogel, 1998).

Fusarium spp. are among the most economically important fungal pathogens and can produce several mycotoxins which able to cause a variety of toxic effects, so we should pay more attention to them.

References

-Abedi-Tizaki, M. and S.K. Sabbagh. 2012. Morphological and molecular identification of *Fusarium* head blight isolates from wheat in north of Iran. Australian Journal of Crop Science, 6(9):1356-1361.

-Burgess, L.W., B.A. Summerell, S. Bullock, K.P. Gott and D. Backhouse. 1994. Laboratory Manual for *Fusarium Research*; University of Sydney: Sydney, Australia.

-Bluhm, B. H., J.E. Flaherty, M.A. Cousin and C.P. Woloshuk. 2002. Multiplex polymerase chain reaction assay for the differential detection of trichothecene- and fumonisin-producing species of *Fusarium* in cornmeal. J Food Prot 65:1955-1961.

-Bluhm, B. H., M.A.Cousin and C.P. Woloshuk. 2004. Multiplex real-time PCR detection of fumonisin producing and trichothecene-producing groups of *Fusarium* species. J Food Prot 67:536-543

-Demeke, T., R.M. Clear, S.K. Patrick and D. Gaba. 2005. Species-specific PCR based assays for the detection of *Fusarium* species and a comparison with the whole seed agar plate method and trichothecene analysis. Intl J Food Microbiol 103:271-284.

-Desjardins, A. E., T. M. Hohn and S.P. McCormick. 1993. Trichothecene biosynthesis in *Fusarium* species: chemistry, genetics, and significance. Microbiol Rev 57:595-604.

-Doohan, F. M., G. Weston, H.N. Rezanoor, D. W.Parry and P. Nicholson. 1999. Development and use of a reverse transcription-PCR Assay to study expression of Tri5 by *Fusarium* Species in vitro and in planta. Appl Environ Microbiol 65:3850-3854.

-Geiser, D. M, J. Gasco, M.M. Kang, S. Mkalowska, I. Veeraraghavan and N. Ward. 2004. *FUSARIUM-IDv.*1.0: a DNA sequence database for identifying. *Fusarium*. Eur J Plant Pathol 110: 473-479.

-Goswami, R.S. and H.C.Kistler.2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Pathol 5:515-525.

-Ismail, M. A., S.I.I.Abdel-Hafez, N.A. Hussein and N.A. Abdel-Hameed. 2015. Contributions to the genus *Fusarium* in Egypt. First Edition. Tmkarpiński publisher, Suchy Las, Poland.

-Hogg, A.C., R.H. Johnston, J.A. Johnston, L. Klouser, K.D. Kephart and A.T. Dyer. 2010. Monitoring *Fusarium* crown rot populations in spring wheat residues using quantitative real-time polymerase chain reaction. *Phytopathology* 100: 49-57.

-Joffe, A. Z. 1974. A Modern system of *Fusarium* taxonomy. Mycopathologia et Mycologia applicata, vol, 53: 201-228.

-Jurado, M., C. Vazquez, S. Marın, V. Sanchis and M.T. Gonzalez Jaen. 2006. PCR-based strategy to detect contamination with mycotoxigenic *Fusarium* species in maize . Systematic and Applied Microbiology 29 :681-689.

-Leslie, J. F. and B.A. Summerell. 2006. The Fusarium Laboratory Manual. Blackwell.,Oxford.

-Matny , O. 2015. *Fusarium* head blight and crown rot on wheat and barley: Losses and health risks. European Journal of Botany, Plant Sciences and Phytology 2(30):27-35.

-Niessen, M. L. and F.F.Vogel. 1998. Group specific PCR-detection of potential trichothecene-producing *Fusarium*-species in pure cultures and cereal samples. Syst. Appl. Microbiol. 21:618–631.

-Nelson, P., T. Toussoun and W. Marasas. 1983. Fusariumspecies: an illustrated manual for identification. Pennsylvania State University Press, University Park, PA. p. 193.

-Nicholson, P., D.R. Simpson, G. Weston, H.N. Rezanoor, A.K. Lees, D. W. Parry and D. Joyce. 1998. Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. Physiol Mol Plant Pathol 53:17-37.

-O'Donnell, K., H.C. Kistler, E. Cigelnik and R.C. Ploetz. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. ProcNatlAcadSci U S A 95:2044-2049.

-Rahjoo, V., J. Zad, M. Javan-Nikkhah, A. MirzadiGoharim, S. Okhovvat, M. and M. R. Bihamta. 2008. Morphological and molecular identification of *Fusarium* isolated from maize ears. Iran J Plant Pathol 90:463-468.

-Scherm, B., V. Balmas, F. Spanu, G. Pani, G. Delogu, M . Pasquali and Q. Migheli. 2013. *Fusarium* culmorum: causal agent of foot and root rot and head blight on wheat. Molecular Plant Pathology 14(4): 323-341.

-Schilling, A. G., M.E. Moller and H.H. Geiger. 1996. Polymerase chain reaction-based assays for species specific detection of *Fusarium culmorum*, *F. graminearum* and *F. avenaceum*. Phytopathology 86:515–522.

-Spanic, V., M. Lemmens and G. Drezner. 2010. Morphological and molecular identification of *Fusarium species* associated with head blight on wheat in East Croatia. Eur J Plant Pathol .128:511-516.

Summerell, B. A. and J.F. Leslie. 2011. Fifty years of *Fusarium*: how could nine species have ever been enough? Fungal Diversity, 50(1) : 135-144.

-Wagacha, J. M. and J.W. Muthomi. 2007. *Fusarium culmorum*: infection process, mechanisms of mycotoxin production and their role in pathogenesis in wheat. Crop Prot. 26: 877-885.

-Wang, H., S.F. Hwang, F. Eudes, K.F. Chang, R.J. Howard and G.D. Turnbull. 2006. Trichothecenes and aggressiveness of *Fusarium graminearum* causing seedling blight and root rot in cereals. Plant Pathol. 55:224-230.
-Zidan, L., A. Skheta, D. Jawdat, and W. Naffaa. Comparison of five DNA extraction methods from some *Fusarium* species. Damascus University Journal for Agricultural Researches. (In press).

N° Ref: 944