Phytoparasitica (2013) 41:241–248
DOI 10.1007/s12600-012-0284-7

Morphological and molecular identification and PCR amplification to determine the toxigenic potential of *Fusarium* spp. isolated from maize ears in southern Poland

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Received: 2 March 2012 / Accepted: 19 December 2012 / Published online: 4 January 2013

Abstract The average amount of precipitation in spring and summer 2010 and 2011 coupled with relatively high temperatures caused massive *Fusarium* spp. infection of maize and yield losses in southern Poland. In order to examine the cause of this disease outbreak, *Fusarium* spp. were isolated and fungal strains were identified based on morphological characters and species-specific PCR assays. A total of 200 maize samples were processed, resulting in the obtention of 71 strains, which belonged to five *Fusarium* species, *F. poae* being the predominant one (74.56%). Other isolates were identified as *F. graminearum*, *F. oxysporum*, *F. verticillioides* and *F. proliferatum*. PCR-based detection of mycotoxin-synthesis-pathway genes was also used to determine the potential of the analyzed strains to produce trichothecenes (DON and NIV) and fumonisins (FUM). Only 14 isolates revealed the potential to produce DON (11 strains) and FUM (3 strains). HPLC analyses of grain samples revealed the presence of DON only – other mycotoxins were not detected. Moreover, 57.1% of potentially mycotoxin-producing isolates indicated the toxicity in a biological test.

Keywords Mycotoxins · Species-specific PCR · *Zea mays* L.

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Introduction

Maize (*Zea mays* L.) has high yield potential and a high content of nutrients, which plays a significant role in animal and human nutrition (Michalski 1999). This makes it a species of great economic importance. Together with wheat and rice it occupies a worldwide leading position in the production of food and animal feed. Maize accounts for 20% of the overall global area planted with cereals, accounting for 30% of global grain production. Currently, the traditional corn grain usage system prevails in Poland: nearly 80% of the harvest is used as feed (Statistical Yearbook of Agriculture 2010).

Maize plants are attacked by various pathogens such as *Fusarium* spp., *Ustilago maydis*, *Sprisorium reiliana* (Xu *et al.* 1999). One of the most commonly and widely studied maize diseases in Poland is *Fusarium* ear rot (Chełkowski *et al.* 1994, 2007;

Pascale *et al.* 2002), which is a major threat to maize production worldwide, mainly due to the mycotoxins-producing ability of *Fusarium* species. The *Fusarium*-mediated mycotoxin contamination is a potential health hazard for humans and animals consuming maize and maize-based products (Goertz *et al.* 2010). The growing period of 2008 was the first year when the hazard assessment of *Fusarium* spp. occurrence was monitored in Poland. Maize ear rot was reported commonly, but its severity was low (Statistical Yearbook of Agriculture 2010).

As the pathogenicity of various *Fusarium* species is diverse, the mycotoxin-production ability may vary even between particular strains (Goswami & Kistler 2005). Rapid and accurate *Fusarium* spp. identification, as well as detection of their mycotoxin production ability, is therefore vital to reduce the harmful effects of the disease (Bayraktar & Dolar 2011).

The amount of precipitation in southern Poland in spring and summer 2010 and 2011 was very high, the average rain amount exceeded significantly the monthly precipitation rates. Heavy rain coupled with relatively high average temperatures promoted growth of *Fusarium* spp. on cereals (Pląskowska 2010), which caused maize yield losses in 2010 and 2011.

The aims of this study were to (i) identify the *Fusarium* species isolated from maize ears in southern Poland in 2010 and 2011 using morphological and molecular methods by species-specific PCR; (ii) determine the genetic potential of *Fusarium* spp. isolates to produce nivalenol, deoxynivalenol and fumonisins; and (iii) evaluate the toxicity of metabolites of the maize-derived strains.

Materials and methods

Fungal isolates Maize grain samples were collected at the end of two growing seasons (October 2010 and 2011) from the experimental fields of University of Agriculture in Cracow located in Prusy, Małopolskie voivodeship (southern Poland). A total of 100 cobs of *Zea mays* were examined each year. Six maize seeds from each cob were surface-sterilized for 1 min with a 1% sodium hypochlorite solution (NaOCl), rinsed twice in sterile distilled water and placed in petri dishes with Potato Dextrose Agar (PDA). The petri dishes were incubated for 5–7 days at 25°C. All *Fusarium* isolates were purified and stored at 4°C for further analyses. Morphological identification of *Fusarium* spp. was carried out according to Leslie & Summerell (2006) and Nelson *et al.* (1983). The following characters of *Fusarium* cultures were assessed by eye and microscopic examination: colony morphology (PDA), macroconidia, microconidia and chlamydospores.

DNA extraction *Fusarium* spp. isolates were cultured on PDA for 7 days. Afterwards, mycelia were scraped into Eppendorf tubes with a sterile spatula. Total DNA was extracted from mycelium of each isolate (about 100 mg wet weight) using Bead-Beat Micro Gravity DNA isolation kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's instructions. The isolation kit contained 1 mm Zirconia/Silica beads which enabled us to grind the mycelium using Retsch MM 400 Mixer Mill (30 Hz, 3 min). The quality and quantity of DNA obtained was assessed using NanoDrop spectrophotometer (Thermo Scientific, USA).

Species-specific PCR Studied isolates were identified with species-specific PCR assay using previously published primer pairs for *F. poae*, *F. oxysporum*, *F. graminearum*, *F. sporotrichioides*, *F. culmorum*, *F. proliferatum* and *F. verticillioides* (Table 1). PCR reactions were performed for all isolates and sterile deionized water served as negative control. Positive control was not applied but all DNA templates gave eventually a product only with a single primer pair. The reaction mixtures of a total volume of 25 μl contained 10×PCR buffer, 1.5 mM of $MgCl_2$, 0.3 μM of each primer, 0.2 mM of dNTPs, 1 U of Taq DNA Polymerase (Thermo Scientific - Fermentas, Canada) and approximately 25 ng of fungal template DNA. PCR amplification was carried out in the Veriti 96-Well Thermal Cycler (Applied Biosystems, Carlsbad, CA, USA) using temperature profiles described by Demeke *et al.* (2005), Koncz *et al.* (2008), Mishra *et al.* (2003), Mulè *et al.* (2004a) Nicholson *et al.* (1998), and Rahjoo *et al.* (2008). The PCR products were visualized by 1×TBE electrophoresis in ethidium-bromide-stained 1% agarose gel.

Molecular analyses of the toxigenic potential of Fusarium spp. isolates The potential of *Fusarium* spp. isolates to produce fumonisin and trichothecenes was determined by the PCR-based molecular analyses

Table 1 Species-specific primers used for the identification of *Fusarium* spp. isolated from maize plants

Primer name	Sequence 5′-3′	Product size (bp)	Target fungi	Source
Fpo-R Fsp-F	CAGCGCACCCCTCAGAGC CGCACGTATAGATGGACAAG	~400	*F. poae* *F. poae, F. sporotrichioides*	Jurado *et al.* 2005
Fsp-R	GTCAGAAGAGACGCATCCGCC	~400	*F. sporotrichioides*	Jurado *et al.* 2005
Fgr-F Fgc-R	GTTGATGGGTAAAAGTGTG CTCTCATATACCCTCCG	~500	*F. graminearum*	Jurado *et al.* 2005
CLPRO1 CLPRO2	TGCATCAGACCACTCAAATCCT GCGAGACCGCCACTAGAT	526	*F. proliferatum*	Mulè *et al.* 2004a
VER1 VER2	CTTCCTGCGATGTTTCTCC AATTGGCCATTGGTATTATATATCTA	578	*F. verticillioides*	Mulè *et al.* 2004b
FOF1 FOR1	ACATACCACTTGTTGCCTCG CGCCAATCAATTTGAGGAACG	~340	*F. oxysporum*	Mishra *et al.* 2003
C51F C51R	ATGGTGAACTCGTCGTGGC CCCTTCTTACGCCAATCTCG	~570	*F. culmorum*	Nicholson *et al.* 1998

using the Tri13F and Tri13DONR, Tri13NIVF and Tri13R, and FUM1F and FUM1R specific primers pairs (Table 2) which target the mycotoxin-synthesis-pathway genes: *Tri13DON* (deoxynivalenol), *Tri13NIV* (nivalenol) and *fum1* (fumonisin), respectively. PCR reactions were performed for all isolates and sterile deionized water served as negative control. Positive control was not used as the quality of all DNA extracts was assessed previously in species-specific PCR. The reaction mixtures of a total volume of 25 μl contained 10×PCR buffer, 2 mM of $MgCl_2$, 0.4 μM of each primer, 0.2 mM of dNTPs, 0.75 U of Taq DNA Polymerase (Thermo Scientific - Fermentas, Burlington, Ontario, Canada) and approximately 50 ng of fungal template DNA. PCR amplification was carried out in the Veriti 96-Well Thermal Cycler (Applied Biosystems) according to temperature profiles described by Lenc *et al.* (2008) and Yazeed *et al.* (2011). The PCR products were visualized by 1×TBE electrophoresis in ethidium-bromide-stained, 1% agarose gel.

Biological tests In order to evaluate the phytotoxic effect of fungal metabolites to plants, the biological tests were performed with 14 *Fusarium* spp. isolates. Only potentially fumonisin- or trichothecene-producing strains were included. The test was performed on 20 maize seeds per isolate according to the procedure described by Mirczink (1957). After incubation for 3, 5 and 10 days, percent germination energy was calculated for each isolate. Isolates were regarded as toxic when the germination energy was decreased by 30% or more as compared with control plants (Bis 2006).

Chemical detection of mycotoxins The studied maize grain samples, from which the potentially mycotoxin-producing *Fusarium* strains were isolated, were analyzed chemically to determine the concentration of deoxynivalenol, nivalenol and B1 and B2 fumonisins. Detection of mycotoxins was carried out at the National Veterinary Research Institute in Pulawy,

Table 2 Primers used for detection of the *Fusarium* spp. potential to produce trichothecenes and fumonisin

Primer name	Sequence 5′-3′	Product size (bp)	Target sequence	Source
FUM1 F FUM1 R	CCATCACAGTGGGACACAGT CGTATCGTCAGCATGATGTA	183	*fum1* gene	Bluhm *et al.* 2004
Tri13F Tri13DONR	CATCATGAGACTTGTKCRAGTTTGGG GCTAGATCGATTGTTGCATTGAG	282	*Tri13DON* gene	Chandler *et al.* 2003
Tri13NIVF Tri13R	CCAAATCCGAAAACCGCA TTGAAAGCTCCAATGTCGTG	312	*Tri13NIV* gene	Chandler *et al.* 2003

Poland, with high performance liquid chromatography (HPLC) assay, following the method described by Plattner (1999).

Results

Morphological and molecular identification A total of 71 strains (27 from the first year of the study) belonging to five *Fusarium* species were isolated from the analyzed maize grain samples. Based on morphological observations, 53 isolates were identified as *F. poae*, ten as *F. graminearum*, five as *F. oxysporum* and two as *F. proliferatum*. Five isolates, however, could not be identified to species level.

Because of incomplete morphological identification, the species-specific PCR analysis with all fungal isolates was performed. The species affiliation of all previously identified strains was confirmed. This method allowed also for identification of the isolates whose species could not be determined using morphological identification only. The expected amplicon sizes were obtained in PCR reactions for *F. graminearum, F. oxysporum, F. poae, F. proliferatum* and *F. verticillioides* (Fig. 1), whereas no isolate was identified as *F. culmorum* or *F. sporotrichioides*. The frequency of species occurrence is presented in Table 3.

Molecular analyses of the toxigenic potential of Fusarium spp. isolates Molecular detection of deoxynivalenol resulted in positive identification of 11 potential DON producers: seven isolates from the first year of the study (five *F. poae* strains, one *F. graminearum* strain and one *F. proliferatum*) and four isolates from the second year (two *F. poae* and two *F. graminearum* isolates). For those isolates the characteristic 282 bp-long fragment was

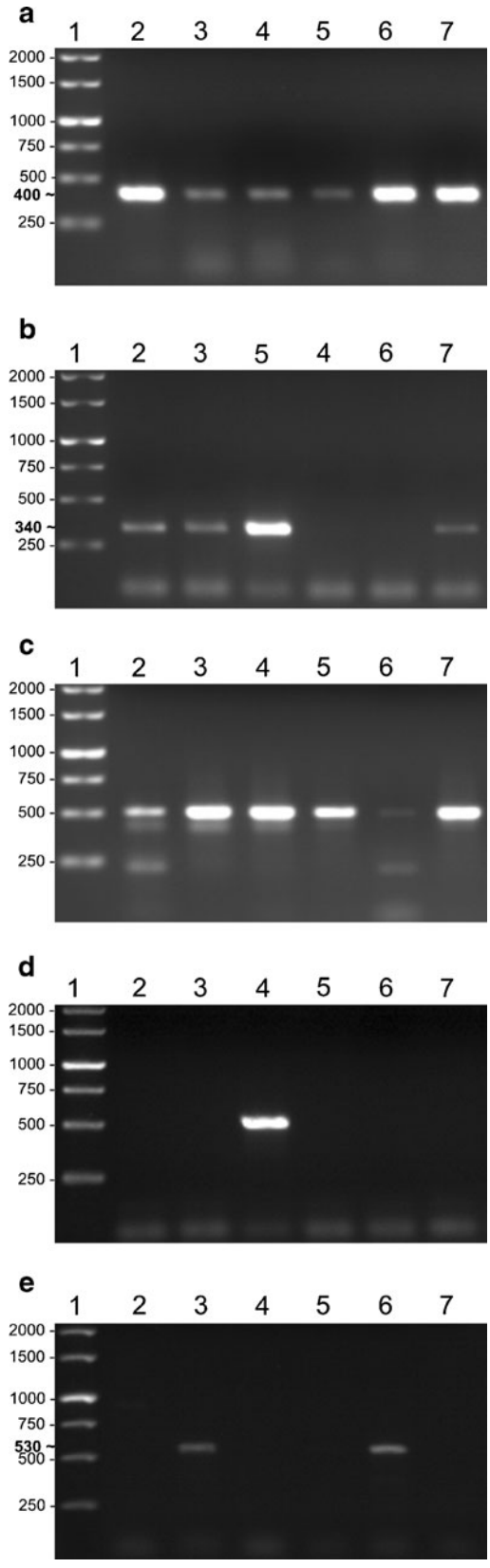

Fig. 1 PCR detection of various *Fusarium* species using species-specific primer sets: **a** Fsp-F/Fpo-R; **b** Fgr-F/Fgc-R; **c** FOF1/FOR1; **d** VER1/2; **e** CLPRO1/2. Lane 1: GeneRuler 1 kb DNA Ladder (ThermoScientific - Fermentas, Canada). Lanes A 2, 3, 4, 5, 6, 7: positively identified *Fusarium poae* isolates. Lanes B 2, 3, 4, 7: positively identified *Fusarium oxysporum* isolates. Lanes C 2, 3, 4, 5, 6, 7: positively identified *Fusarium graminearum* isolates. Lane D 4: positively identified *Fusarium verticillioides* isolate. Lanes E 3, 6: positively identified *Fusarium proliferatum* isolates

Table 3 Overall frequencies and percentage of various *Fusarium* species isolated from maize seeds in Prusy, southern Poland. Frequencies and percentage of potentially toxigenic (DON and FUM) isolates

Fungal species	Year			
	2010		2011	
	No. of isolates (percentage)	No. of potentially toxigenic isolates (percentage)	No. of isolates (percentage)	No. of potentially toxigenic isolates (percentage)
F. poae	21 (29.57)	6 (8.45)	32 (45.07)	2 (2.82)
F. graminearum	3 (4.23)	1 (1.41)	7 (9.86)	2 (2.82)
F. oxysporum	0 (0)	0 (0)	5 (7.04)	0 (0)
F. verticillioides	1 (1.41)	1 (1.41)	0 (0)	0 (0)
F. proliferatum	2 (2.82)	2 (2.82)	0 (0)	0 (0)
Total number/percentage of isolates	27 (38.03)	10 (14.08)	44 (61.97)	4 (5.63)

generated (Fig. 2a). Detection of the second trichothecene, nivalenol, gave no positive results, as the expected product (312 bp) was not amplified for any of the studied isolates.

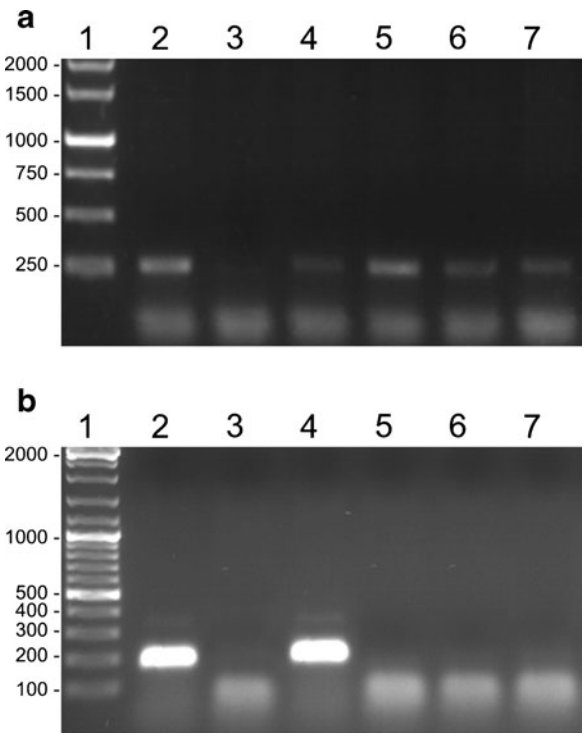

Fig. 2 PCR detection of DON and fumonisin production potential indicated by the presence of Tri13DON and fum1 markers: **a** Tri13F/Tri13DONR; **b** FUM1 F/FUM1 R. Lane 1: **a** GeneRuler 1 kb DNA Ladder; **b** GeneRuler DNA Ladder Mix, 100–10,000 bp Ladder (ThermoScientific - Fermentas, Canada). Lanes A 2, 4, 5, 6, 7: positively identified presence of Tri13-DON marker. Lanes B 2, 4: positively identified presence of fum1 marker

Detection of potential fumonisin producers was positive for three *Fusarium* spp. isolates from the first year of the study (one *F. verticillioides,* one *F. poae* and one *F. proliferatum* strain) only; the amplification yielded the expected product (size 183 bp) (Fig. 2b). No successful amplification was observed among *Fusarium* spp. strains obtained in 2011.

Frequencies and percentage of potential mycotoxin producing strains are given in Table 3.

Biological tests Biological tests were performed in order to determine whether fungal metabolites of 14 strains, whose mycotoxin-synthesis potential was revealed by the described PCR methods, exhibit toxicity to germinating maize seeds. Among the tested strains, metabolites of eight (57.1%) turned out to be toxic, while the inhibiting effect of six of them (42.9%) did not reach the 30% threshold (Table 4). The inhibition of germination energy caused by the studied *Fusarium* spp. strains ranged from 10% to 65%.

Chemical detection of mycotoxins The HPLC analysis of maize grain samples showed varied deoxynivalenol concentration, oscillating around 1,000 $\mu g \times kg^{-1}$. The concentration of DON ranged from 1,327 to 1,055 $\mu g \times kg^{-1}$. Only one sample contained a lower amount of DON – 694 $\mu g \times kg^{-1}$. The other analyzed mycotoxins (nivalenol, fumonisin B1 and B2) were not detected.

Table 4 The results obtained after 10 days of biological tests performed on 14 potentially mycotoxin-producing *Fusarium* spp. strains

Number of a strain (mycotoxin potential)	Species	No. of germinated seeds per 20 [inhibited germination (%)]	Toxic [+]/ non-toxic [−]
1 (FUM)	*F. verticillioides*	11 [45]	+
2 (FUM)	*F. poae*	9 [55]	+
3 (FUM)	*F. proliferatum*	18 [10]	−
4 (DON)	*F. poae*	19 [5]	−
5 (DON)	*F. graminearum*	10 [50]	−
6 (DON)	*F. poae*	16 [20]	−
7 (DON)	*F. poae*	18 [10]	−
8 (DON)	*F. graminearum*	11 [45]	+
9 (DON)	*F. poae*	15 [25]	−
10 (DON)	*F. poae*	13 [35]	+
11 (DON)	*F. poae*	7 [65]	+
12 (DON)	*F. proliferatum*	18 [10]	−
13 (DON)	*F. graminearum*	11 [45]	+
14 (DON)	*F. poae*	12 [40]	+
C-0	control	20	−

Discussion

This research was undertaken mainly to identify the cause of recently observed maize fusariosis in southern Poland, which was probably affected by increased rainfall rates in spring and summer of 2010 and 2011. *Fusarium* species-related diseases are important factors that decrease maize yields (Czembor *et al.* 2010); their significance is greater, since they may affect human or animal health due to mycotoxin synthesis ability (Goertz *et al.* 2010). Mycotoxin synthesis potential may, however, vary between strains, irrespective of the species (Goswami & Kistler 2005). Knowledge of the species composition and, what is more important, mycotoxin synthesis potential of various *Fusarium* spp. strains, is therefore needed to estimate the risk of food and feed mycotoxin contamination.

The results obtained in this study indicated that *F. poae* was one of the most frequently isolated species (74.64%), confirming other reports that this species is one of the most often isolated *Fusarium* spp. pathogens, not only in Poland, but also in other countries (*e.g.* Argentina, Austria, Canada, England, Germany, Hungary, Ireland, Slovakia, Switzerland and Wales) (Stenglein 2009). Such a wide *F. poae* occurrence is unfavorable, as this species belongs to the causal agents of *Fusarium* ear rot (Chełkowski *et al.* 2007), which is one of the most important maize diseases—decreasing yield and affecting grain quality and feed value of the grain (Czembor *et al.* 2010; Rahjoo *et al.* 2008).

Haratian *et al.* (2008) applied the positive-negative PCR assay based on the Tri13 gene to identify the genetic potential of DON and NIV production of *F. graminearum*. In this study, only 11 *Fusarium* spp. were found to be potentially deoxynivalenol-producing strains, as they gave positive amplification of 282 bp product with Tri13F and Tri13DONR primers. This indicates relatively rare deoxynivalenol production potential: 15.5% of all analyzed isolates. This is, however, much more than was detected in similar studies performed in Poland by Lenc *et al.* (2008), who did not find *Fusarium* spp. strains with the potential to produce DON. On the other hand, Burlakoti *et al.* (2008) in their TRI-based PCR assays, revealed that all studied strains had the DON marker.

The potential to synthesize the second trichothecene, *viz.,* nivalenol was not detected; the NIV-specific Tri13 primers Tri13NIVF and Tri13R did not amplify the expected 312 bp-long product (which would have indicated the nivalenol-synthesis ability) among the studied *Fusarium* isolates. Similar results were reported by Pasquali *et al.* (2010), who did not find the potential NIV-producers among *F. poae* isolates derived from wheat harvested in Luxembourg. In other species detected by these authors, the NIV chemotype was also sporadically detected (representing only 5.8% of the total population). Haratian *et al.* (2008) revealed that both DON and NIV *Fusarium* spp. chemotypes existed in Iran, but NIV producers were more frequently reported.

The synthesis ability of the third considered mycotoxin, *viz.,* fumonisin among *Fusarium* spp. isolates was analyzed in accordance with the PCR-based approach with FUM1-F and FUM1-R primer pair introduced by Sreenivasa *et al.* (2008). In this study, only three potential fumonisin producers could be identified. This result is surprising, as it is in contradiction with the data of Sreenivasa *et al.* (2008) and Yazeed *et al.* (2011), in which 53 of 64 plant-derived isolates and all of the 21 feed-samples-derived isolates, respectively, were identified as fumonisin producers.

The results of the biological test are similar to the ones obtained by Bis (2006) in the analysis of potentially toxicogenic fungal strains isolated from soils in Cracow and its surroundings, where only 60% of the isolated strains could have been considered as toxic. *Fusarium graminearum* isolated from soil near a cement plant in Cracow appeared to be the most phytotoxic strain in that study. This strain caused 70% inhibition of germination energy of the tested plant seeds.

The last part of the study included the HPLC detection of deoxynivalenol, nivalenol and fumonisins. Out of three analyzed mycotoxins, only deoxynivalenol was detected. Nonetheless, the amounts of DON do not exceed the maximum levels according to the EC Regulation No. 1126/2007 L 255/14 regarding deoxynivalenol concentration in unprocessed maize. The results obtained from the chemical analyses confirm the results of the PCR amplification with primers targeting deoxynivalenol-synthesis-pathway genes, as several *Fusarium* spp. strains revealed the potential to produce this mycotoxin. Fumonisin contamination of grain samples has not been confirmed in HPLC detection, although three strains were identified as potential fumonisin producers in the PCR approach, and metabolites of two of them have proven to have an inhibiting effect on germinating maize seeds. These results are consistent with those obtained by Wang *et al.* (2010), who indicated in their study of *Fusarium* spp. isolated from asparagus that, although *Fusarium* spp. isolates containing FUM genes produced fumonisins in cultures, no fumonisin contamination was detected in the analyzed asparagus samples. This may be explained by the fact that although the isolates have the genetic potential to produce mycotoxins, the actual production of these secondary metabolites is a very complex process and may be affected by a number of factors, particularly by environmental conditions, *e.g.* temperature, oxygen tension, but also by pH of a medium, in which the fungus grows (Miller 2001).

The results obtained in this study indicate that the recent disease outbreak in maize crops in spring and summer of 2010 was caused mostly by *Fusarium poae*, although other species could also be isolated. A similar situation was observed in 2011, when this species was also the most frequently detected. Still, the mycotoxin-synthesis potential was relatively rare among the isolated strains. Moreover, mycotoxin contamination of grain samples, or inhibiting effect on germinating seeds, has not been observed in all of the potentially toxigenic strains. This means that toxin synthesis, even if possible, is triggered by additional external or internal factors. An interesting factor is that the results of the HPLC analysis and biological tests are not fully congruent, as fumonisin has not been detected in grain samples from which were isolated two potential fumonisin producers with an inhibiting effect on germinating seeds. This indicates that in vitro culturing may affect mycotoxin synthesis which would not have been produced in vivo, or that the concentration of the potential fumonisin producers would not have reached the detection threshold.

Acknowledgments This research was supported by grant No. 4102 funded by the Faculty of Agriculture and Economics, University of Agriculture in Cracow.

References

Bayraktar, H., & Dolar, F. S. (2011). Molecular identification and genetic diversity of *Fusarium* species associated with onion fields in Turkey. *Journal of Phytopathology, 159*, 28–34.

Bis, H. (2006). Ability to produce mycotoxins by fungi isolated from soils in Cracow and the surroundings. *Scientific Journal of Wrocław University of Environmental and Life Sciences – Agriculture, 546*, 43–50.

Bluhm, B. M., Cousin, M. A., & Woloshuk, C. P. (2004). Multiplex real-time PCR detection of fumonisin producing and trichothecene producing groups of *Fusarium* species. *Journal of Food Protection, 3*, 536–543.

Burlakoti, R. R., Ali, S., Secor, G. A., Neate, S. M., McMullen, M. P., & Adhikari, T. B. (2008). Comparative mycotoxin profiles of *Gibberella zeae* populations from barley, wheat, potatoes and sugar beets. *Applied and Environmental Microbiology, 74*, 6513–6520.

Chandler, E. A., Simpson, D. R., Thomsett, M. A., & Nicholson, P. (2003). Development of PCR assays to *Tri*7and *Tri*13 trichothecene biosynthetic genes, and characterisation of chemotypes of *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium cerealis*. *Physiological and Molecular Plant Pathology, 62*, 355–367.

Chełkowski, J., Lew, H., & Pettersson, H. (1994). *Fusarium poae* (Peck) Wollenw. – Occurrence in maize ears, nivalenol production and mycotoxin accumulation in cobs. *Mycotoxin Research, 10*, 116–120.

Chełkowski, J., Ritieni, A., Wiśniewska, H., Mulè, G., & Logrieco, A. (2007). Occurrence of toxic hexadepsipeptides

in preharvest maize ear rot infected by *Fusarium poae* in Poland. *Journal of Phytopathology, 155*, 8–12.

Commission Regulation (EC) No. 1126/2007 (2007). Amending Regulation (EC) No. 1881/2006 setting maximum levels for certain contaminants in foodstuff as regards *Fusarium* toxins in maize and maize products, L 255/14.

Czembor, E., Adamczyk, J., Posta, K., István, S., Oldenburg, E., & Schürch, S. (2010). Prevention of ear rots due to *Fusarium* spp. on maize and mycotoxin accumulation. ENDURE Maize Case Study – Guide Number 3.

Demeke, T., Clearm, R. M., Partick, S. K., & Gaba, D. (2005). Species-specific PCR-based assays for the identification of *Fusarium* species and a comparison with the whole seed agar plate method and trichothecene analysis. *International Journal of Food Microbiology, 103*, 271–284.

Goertz, A., Zuehlkem, S., Spiteller, M., Steiner, U., Dehne, H. W., Waalwijk, C., *et al.* (2010). *Fusarium* species and mycotoxin profiles on commercial maize hybrids in Germany. *European Journal of Plant Pathology, 128*, 101–111.

Goswami, R. S., & Kistler, H. C. (2005). Pathogenicity and *in planta* mycotoxin accumulation among members of the *Fusarium graminearum* species complex on wheat and rice. *Phytopathology, 95*, 1397–1404.

Haratian, M., Sharifnabi, B., Alizadeh, A., & Safaie, N. (2008). PCR analysis of the Tri13 gene to determine the genetic potential of *Fusarium graminearum* isolates from Iran to produce nivalenol and deoxynivalenol. *Mycopathologia, 166*, 109–116.

Jurado, M., Vázquez, C., Patiño, B., & González-Jaén, M. T. (2005). PCR detection assays for the trichothecene-producing species *Fusarium graminearum, Fusarium culmorum, Fusarium poae, Fusarium equiseti* and *Fusarium sporotrichioides*. *Systematic and Applied Microbiology, 28*, 562–568.

Koncz, Z. S., Magyar, D., Naar, Z., Kiss, A., & Szecsi, A. (2008). PCR-based assays for the identification of *Fusarium* spp. originating from wheat grain. *Cereal Research Communications, 36*, 631–636.

Lenc, L., Łukanowski, A., & Sadowski, C. (2008). The use of PCR amplification in determining the toxigenic potential of *Fusarium sambucinum* and *F. solani* isolated from potato tubers with symptoms of dry rot. *Phytopathologia Polonica, 48*, 12–23.

Leslie, J. F., & Summerell, A. B. (2006). *The Fusarium laboratory manual*. Ames, IA, USA: Blackwell Publishing Professional.

Michalski, T. (1999). The healthiness of spring cereals depending on sowing density. *Progress in Plant Protection, 39*, 759–762.

Miller, J. D. (2001). Factors that affect the occurrence of fumonisin. *Environmental Health Perspectives, 109*, 321–324.

Mirczink, T. G. (1957). O gribach obustwliwajuszczich toksicznost diernowo podzolistnoj poczwy rozlicznoj stiepieni okulturiennosti. About mycotoxigenic fungi isolated from humus-podzolic soils of varying degrees of agricultural transformation [in Russian]. *Mikrobiologia, 26*, 78–86.

Mishra, P. K., Fox, R. T. V., & Culham, A. (2003). Development of a PCR-based assay for rapid and reliable identification of pathogenic Fusaria. *FEMS Microbiology Letters, 218*, 329–332.

Mulè, G., Susca, A., Stea, G., & Moretti, A. (2004a). Specific detection of the toxigenic species *F. proliferatum* and *F. oxysporum* from asparagus plants using primers based on calmodulin gene sequences. *FEMS Microbiology Letters, 230*, 235–240.

Mulè, G., Susca, A., Stea, G., & Moretti, A. (2004b). A species-specific PCR assay based on the calmodulin partial gene for identification of *Fusarium verticillioides, F. proliferatum* and *F. subglutinans*. *European Journal of Plant Pathology, 110*, 495–502.

Nelson, P. E., Toussoun, T. A., & Marasas, W. F. O. (1983). *Fusarium species. An illustrated manual for identification*. University Park, PA, USA: The Pennsylvania State University Press.

Nicholson, P., Simpson, D. R., Weston, G., Rezanoor, H. N., Lees, A. K., Parry, D. W., *et al.* (1998). Detection and quantification of *Fusarium culmorum* and *Fusarium graminearum* in cereals using PCR assays. *Physiological and Molecular Plant Pathology, 53*, 17–37.

Pascale, M., Visconti, A., & Chełkowski, J. (2002). Ear rot susceptibility and mycotoxin contamination of maize hybrids inoculated with *Fusarium* species under field conditions. *European Journal of Plant Pathology, 108*, 645–651.

Pasquali, M., Giraud, F., Brochot, C., Cocco, E., Hoffman, L., & Bohn, T. (2010). Genetic Fusarium chemotyping as a useful tool for predicting novalenol contamination in winter wheat. *International Journal of Food Microbiology, 137*, 246–253.

Pląskowska, E. (2010). Characteristics and taxonomy of *Fusarium* fungi. *Mikologia Lekarska (Medical Mycology), 17*, 172–176.

Plattner, R. D. (1999). HPLC/MS analysis of Fusarium mycotoxins, fumonisins and deoxynivalenol. *Natural Toxins, 7*, 365–370.

Rahjoo, V., Zad, J., Javan-Nikkhah, M., Mirzadi Gohari, A., Okhorvat, S. M., Bihamta, M. R., *et al.* (2008). Morphological and molecular identification of *Fusarium* isolated from maize ears in Iran. *Journal of Plant Pathology, 90*, 463–468.

Sreenivasa, M. Y., Dass, R. S., Charith Raj, A. P., & Janardhana, G. R. (2008). PCR method for the detection of genus *Fusarium* and fumonisin-producing isolates from freshly harvested sorghum grains grown in Karnataka, India. *Journal of Food Safety, 28*, 236–247.

Statistical Yearbook of Agriculture. (2010). Warsaw, Poland: Central Statistical Office.

Stenglein, S. A. (2009). *Fusarium poae*: a pathogen that needs more attention. *Journal of Plant Pathology, 91*, 25–36.

Wang, J., Wang, X., Zhou, Y., Du, L., & Wang, Q. (2010). Fumonisin detection and analysis of potential fumonisin-producing *Fusarium* spp. in asparagus (*Asparagus officinalis* L.) in Zhejiang province of China. *Journal of the Science of Food and Agriculture, 90*, 836–842.

Xu, M. L., Melchinger, A. E., & Lübberstedt, T. (1999). Species-specific detection of the maize pathogens *Sporisorium reiliana* and *Ustilago maydis* by dot blot hybridization and PCR-based assays. *Plant Pathology, 83*, 390–395.

Yazeed, H. A. E., Hassan, A., Moghaieb, R. E. A., Hamed, M., & Refai, M. (2011). Molecular detection of fumonisin-producing *Fusarium* species in animal feeds using polymerase chain reaction (PCR). *Journal of Applied Sciences Research, 7*, 420–427.