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MOLECULAR ANALYSIS OF *TRI5* GENE PRESENCE AND MYCOTOXIGENIC ABILITIES OF *FUSARIUM LANGSETHIAE*

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Abstract

Winter wheat kernels originated from four localities in Poland were analyzed for colonization by *Fusarium langsethiae* – a species not described in Poland so far. Its presence was confirmed in 27 samples. The fungus occurred on 0.5 to 3.5% kernels. The presence of *Tri*5 gene in nine strains of *F. langsethiae* was tested with PCR assay. The results confirmed that all of them were potentially able to produce the secondary metabolites from trichothecene group. Further detailed analyses of four *F. langsethiae* strains showed the production of T-2 and HT-2 toxins at concentrations 44.5-4580 ppb and 1780-8960 ppb, respectively.

Key words: *Fusarium langsethiae*, PCR, mycotoxins, wheat kernels

Introduction

Cereal diseases caused by *Fusaria*, one of the most dangerous pathogens' group of these plants, result in serious losses of yield and its quality. *Fusarium* spp. cause very serious problems in wheat production. An important feature of these fungi, which makes them so popular in the environment, is their wide-world occurrence and high competitiveness and ability to co-exist with other pathogens (Michuta-Grimm and Foster 1989, Zhuping 1994, Sayler 1998). These fungi make a potential threat also in Poland, mainly in regions with higher rainfalls, especially during anthesis (Chełkowski 1994, Kiecana and Perkowski 1998, Łukanowski and Sadowski 2002). Identification of disease causal agent is difficult because of high morphological similarity of *Fusarium* species and their occurrence as disease complex on the same host. Besides, *Fusaria* are polyphagous, and *formae speciales* of particular species often are not distinguishable on the basis of morphological features.

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Fusaria are known not only as fungal plant pathogens, but also as producers of a wide variety of mycotoxins contaminating kernels and consequently food and feed, posing a threat to human and animal health. Each species produces a more or less characteristic pattern of toxins, but there is also variation observed between strains within the same species (Marasas 1991).

The trichothecenes belong to the most hazardous metabolites produced by *Fusarium* species, mainly those belonging to *Sporotrichiella* section, like *F. sporotrichioides* (Placinta et al. 1999, Torp et al. 2000, Brown et al. 2001). Type A of trichothecenes, especially T-2 toxin and HT-2 toxin, are regularly detected in cereal samples (Toxigenic... 1984). Potential ability of *Fusarium* species for production of trichothecenes depends on the presence of *Tri*5 gene encoding trichodiene synthase, an enzyme catalyzing formation of trichodiene – the first product in biosynthetic pathway of trichothecenes (Edwards et al. 2001).

During research on contamination of cereal grain with mycotoxins in Norway, it was found that T-2 and HT-2 toxins were synthesized mainly by an unknown *Fusarium* species with powdery appearance (Kosiak et al. 1997, Langseth and Rundberget 1999). After detailed morphological and molecular surveys it was described as a new species – *Fusarium langsethiae* (Torp and Nirenberg 2004). Morphologically it resembles *F. poae* but from the toxicological point of view it was similar to *F. sporotrichioides* (Schmidt et al. 2004).

So far, there were no reports on occurrence of *F. langsethiae* in Poland. Mycological analyses of wheat kernels collected from fields located in Osiny near Puławy (south-east Poland), conducted at our Department in 2006 showed its presence in two kernel samples.

The aim of the studies was an assessment of (i) the presence of *F. langsethiae* in higher number of wheat kernel samples collected from various regions of Poland and (ii) abilities of *F. langsethiae* to produce trichothecenes.

Materials and methods

The objects of the study were kernel samples of 41 cultivars of winter wheat grown in different regions of Poland in 2005/06 cropping season. Altogether 76 samples were analyzed, collected from (i) cultivar survey located in Osiny near Puławy (Institute of Soil Science and Plant Cultivation – National Research Institute), (ii) cultivar survey of ProCam company established in Experimental Station for Cultivar Testing (ESCT) in Radostowo near Tczew (central-north Poland), (iii) two commercial fields in Kujavia and Pomerania province (central-north Poland) as well as fungicidal experiment of ProCam (ESCT, Radostowo) established on cv. 'Ritmo' consisting of 20 combinations, R1–R20 (Table 1).

The tested kernels were washed for 30 min in running tap water, next trice in sterile deionised water and placed onto Petri dishes with potato dextrose agar medium (pH 5.5). After 7–10 days of incubation at 22°C outgrowing fungal colonies were identified with mycological keys (Gilman 1957, Booth 1971, Kwaśna et al.

Table 1

Colonization of winter wheat kernels by *Fusarium* spp.

Localization	No./origin of samples	No. of samples/ /cultivars with *F. langsethiae*	Kernels with *F. langsethiae* (%)	Kernels with *Fusarium* spp. (%)
Radostowo (Powiśle)	27 cultivars	8, including:		
		'Bogatka'	1.5	18.0
		'Milwus'	1.0	11.1
		'Nadobna'	0.5	17.7
		'Pegassos'	1.0	12.0
		'Ritmo'	1.5	21.8
		'Sakwa'	1.8	17.8
		'Turnia'	0.5	10.5
		'Zyta'	1.8	23.6
Radostowo (cv. 'Ritmo')	20 combinations of fungicidal protection R1–R20	15, including:		
		R1	0.8	28.8
		R2	2.0	34.0
		R3	1.2	25.2
		R4	1.0	22.0
		R6	0.8	34.5
		R7	0.5	28.5
		R10	1.0	38.8
		R12	3.5	30.2
		R14	1.5	27.0
		R15	1.0	21.3
		R16	1.8	22.8
		R17	1.0	35.0
		R18	0.8	21.0
		R19	1.0	28.2
		R20	1.2	32.2
Osiny (near Puławy)	27 samples	2, including:		
		'Zyta'	1.5	6.0
		'Kobra'	1.0	1.0
Minikowo (Kujavia and Pomerania province)	'Trend'	1: 'Trend'	3.5	9.5
Sobiejuchy (Kujavia and Pomerania province)	'Smuga'	1: 'Smuga'	1.0	1.5

R – combinations of spraying with various fungicides.

1991). Isolates morphologically resembling *F. poae*, with powdery appearance were initially identified as *F. langsethiae* (Schmidt et al. 2004, Torp and Nirenberg 2004). The final species confirmation was carried out with PCR reactions that produced species-specific 310 bp amplicon (Wilson et al. 2004).

The potential ability of nine *F. langsethiae* isolates to produce secondary metabolites from trichothecene group was also assessed (Table 2). DNA extraction was conducted according to methodology described by Doyle and Doyle (1990). PCR assay was carried out using optimized thermal profile and SCAR primers (Edwards et al. 2001) expecting amplification of 260 bp *Tri*5 gene fragment localized in the centre of a 25-kb cluster of co-regulated trichothecene biosynthetic genes (Desjardins and Proctor 2007). Amplicons were separated in 1.5% agarose gel (80 V for 2 h) stained with ethidium bromide and results were archived as graphic file using gel documentation system (Vilber Lourmat, BioCapt software).

Table 2

Origin of *Fusarium langsethiae* isolates used to study the potential mycotoxigenic ability

Isolate	Region	Cultivar
6	Minikowo	'Trend'
7	Minikowo	'Trend'
8	Minikowo	'Trend'
10	Sobiejuchy	'Smuga'
22	Radostowo	'Turnia'
23-1	Radostowo	'Ritmo'
23-2	Radostowo	'Ritmo'
R2	Radostowo	'Ritmo'
R14	Radostowo	'Ritmo'

Four *F. langsethiae* isolates (Fl-8, Fl-10, Fl-R14 and Fl-22) were tested for mycotoxin production: DON and its derivatives (3AcDON, 15AcDON), NIV, T-2 toxin and HT-2 toxin on sterile rice grain (40% water content) in 250 ml Erlenmeyer flasks. Experiment was carried out for four weeks at 23°C, and then mycotoxin concentration was determined with HPLC-MS/MS method (Department of Experimental Biology, Kazimierz Wielki University, Bydgoszcz).

Results

Fusarium spp. occurred on 1.5% up to 38.8% of kernels. The presence of *F. langsethiae* was noted in 27 out of 76 wheat kernel samples. The share of kernels settled by *F. langsethiae* was fairly low (0.5–3.5%) (Table 1) and thus the expected relationship between the occurrence of this species, applied fungicides and wheat cultivars could have not been statistically analyzed.

Phot. 1. Analysis of potential mycotoxigenic ability of nine *Fusarium langsethiae* strains to produce trichothecenes. KN – negative control (reaction mixture without DNA) (photo by A. Łukanowski)

Molecular assay (PCR) confirmed potential ability of all examined *F. langsethiae* isolates (Table 2) to produce trichothecenes (Phot. 1).

Out of the nine *F. langsethiae* isolates presented in Table 2, four were selected for further examination. Detailed chemical analysis of trichothecenes synthesized by the four isolates clearly showed that they produced only two toxins belonging to group A: T-2 and HT-2. Mycotoxin content varied and ranged from 44.5 ppb for isolate no. 10 up to 4580 ppb for isolate R-14 (Table 3).

Discussion

The fact that *F. langsethiae* was present in more than one third of the winter wheat kernel samples investigated, may point to its potential importance in the *Fusarium* complex and as a mycotoxin producer.

Table 3

Mycotoxins produced by *Fusarium langsethiae*

Isolate	Toxin	Concentration (ppb)
8	T-2	404
	HT-2	5 590
	deoxynivalenol	nd
	nivalenol	nd
	3-acetyldeoxynivalenol	nd
	15-acetyldeoxynivalenol	nd
10	T-2	44.5
	HT-2	1 780
	deoxynivalenol	nd
	nivalenol	nd
	3-acetyldeoxynivalenol	nd
	15-acetyldeoxynivalenol	nd
22	T-2	161
	HT-2	3 540
	deoxynivalenol	nd
	nivalenol	nd
	3-acetyldeoxynivalenol	nd
	15-acetyldeoxynivalenol	nd
R14	T-2	4 580
	HT-2	8 960
	deoxynivalenol	nd
	nivalenol	nd
	3-acetyldeoxynivalenol	nd
	15-acetyldeoxynivalenol	nd

nd – not detected.

The production of only two toxins belonging to group A: T-2 and HT-2 by the four *F. langsethiae* isolates examined in detail confirms reports of Schmidt et al. (2004), who consider *F. langsethiae* one of the main T-2 and HT-2 toxin producers.

Considerable variation of mycotoxin amount produced by the four isolates (ranging from 44.5 ppb for isolate no. 10 up to 4580 ppb for isolate R-14), is in accordance with reports of Sugiura et al. (1993) and Langseth et al. (1999) who found high variation of particular toxins concentration within isolates of the same species.

Metabolite profile may be an important parameter in the taxonomy of fungi from the *Fusarium* genus, but determination of full spectrum of mycotoxins produced by each individual species requires further more detailed research on the topic.

Streszczenie

MOLEKULARNA ANALIZA WYSTĘPOWANIA GENU *TRI5* I ZDOLNOŚCI MIKOTOKSYNOTWÓRCZYCH *FUSARIUM LANGSETHIAE*

Analizowano zasiedlenie prób ziarna pszenicy ozimej pochodzących z różnych rejonów kraju przez nieopisywany dotąd w Polsce gatunek – *Fusarium langsethiae*. Jego obecność stwierdzono w 27 spośród 76 przebadanych prób. Zasiedlenie ziarna przez ten gatunek było stosunkowo niewielkie i wynosiło od 0,5 do 3,5%. Za pomocą testu PCR określano obecność genu *Tri*5 i potwierdzono zdolność badanych izolatów *F. langsethiae* do tworzenia mikotoksyn z grupy trichotecyn. Szczegółowe analizy chemiczne czterech wybranych izolatów grzyba wykazały, że tworzyły one toksyny T-2 i HT-2 w bardzo zróżnicowanych ilościach (44,5-4580 ppb w przypadku T-2 oraz 1780-8960 ppb w przypadku HT-2).

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