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THE TOXIGENIC POTENTIAL OF *FUSARIUM POAE* ORIGINATED FROM WHEAT

ABSTRACT: Eleven isolates of *F. poae*, originated from wheat grain at 9 locations mainly in Vojvodina, were encompassed by the present study. The greatest number of samples was collected in 2005, in which the climatic conditions favoured a more intensive occurrence of *Fusarium* ear blight of wheat. In order to determine toxicological potential of this species, cultures of the selected isolates were grown in liquid media (GPY and SPY) on a rotary shaker (180 revolutions min^{-1}), at room temperature (21—26°C) for three days. Crude toxins were isolated from liquid culture filtrates of isolates by the use of ethyl acetate, while quantification of mycotoxins was done by the thin layer chromatography method. A liquid culture of the isolate GZ-LES (*F. graminearum*) was used as a control for the evaluation of the zearalenone biosynthesis potential. On the other hand, the liquid culture of the isolate KF-38/1 (*F. sporotrichioides*) was used as a control for both type-A trichothecenes (T-2 toxin and diacetoxyscirpenol — DAS).

The obtained results show that *F. poae*, in contrast to *F. graminearum*, has no potential for the zearalenone biosynthesis. The presence of DAS was determined only in one isolate of *F. poae* (MRIZP-666), and in the control isolate of *F. sporotrichioides* (KF-38/1/R), that were grown in the GPY liquid medium. The T-2 toxin was detected in the isolate MRIZP-666, grown in both media, and in the isolates MRIZP-37 and MRIZP-860, cultured in the GPY and SPY liquid medium, respectively. The control culture KF-38/1/R (*F. sporotrichioides*) produced the T-2 toxin at the concentration of 4,000 μg L^{-1}. According to the gained information, it can be concluded that the potential of *F. poae* for the type-A trichothecene biosynthesis was low, as the concentration of DAS or T-2 toxin did not exceed 80 μg L^{-1} or 240 μg L^{-1}, respectively.

KEY WORDS: diacetoxyscirpenol, *F. poae*, *in vitro* biosynthesis, T-2 toxin, wheat

INTRODUCTION

The occurrence of *Fusarium* head blight of stronger intensity in wheat was recorded not only in Europe, including Serbia (Lević et al., 2004), but worldwide during the last decade of the 20th century. The disease resulted in a

significant economic damage, due to the grain yield reduction, in a quality loss, due to shrunk grain, and in contamination with mycotoxins.

An enormous number of species of the genus *Fusarium*, including *F. poae* (Peck) Wollenw, was isolated from *Fusarium* damaged wheat grain (Waalwijk, 2002). Although a high percentage of *Fusarium poae* was isolated from the grain of wheat, barley and oats in certain years, its role in the aetiology of *Fusarium* head blight had not been yet completely clarified (Kestemont et al., 2002; Kryuchkova et al., 2002; Lew et al., 2001; Hornok and Toth, 2001; Hysek et al., 2000).

It is most often stated that only *F. graminearum* Schwabe and *F. culmorum* (W. G. Smith) Sacc. are important for the aetiology of *Fusarium* head blight (Teich, 1989). Parry et al. (1995) and Waalwijk (2002) have an opinion that *Fusarium* head blight could be caused by four species — two previously stated, *F. avenaceum* (Fr.) Sacc. and *F. poae*. Furthermore, in England, *F. poae* has been very often isolated from chaff spots, although the connection between its occurrence and head blight has not been confirmed (Nicholson et al., 1997).

The species *F. poae* is important from the toxicological aspect as it biosynthesises a great number of mycotoxins, such as: diacetoxyscirpenol (DAS), monoacetoxyscirpenol, nivalenol, fusarenone-X, T-2 toxin, HT-2 toxin, T-2 triol, T-2 tetraol, deoxysinivalenol, neosolaniol, beauvericin and enniatines (BEA) (Bottalico, 1998; Torp and Langseth, 1999; Nicholson et al., 2004; Chełkowski et al., 2007). Out of the stated mycotoxins, type-A trichothecenes (T-2 toxin and DAS), produced by these species, are the most important.

In Italy, it was determined that two main *Fusarium* species isolated from wheat, *F. poae* and *F. sporotrichioides* Sherb., biosynthesised the T-2 toxin and DAS in the amount that ranged from 6,200 to 120,000 ppb (Criseo et al., 1989). According to the results obtained by Gagkaeva et al. (2006), *F. poae* synthesises more DAS than *F. sporotrichioides*, 400 ng mL^{-1} vs. 62 ng mL^{-1}, while *F. sporotrichioides* produces more T-2 toxins (75,860 ng mL^{-1}) than *F. poae* does (158 ng mL^{-1}). Besides the type-A trichothecenes, it was determined that the species *F. poae*, originated from *Fusarium* damaged wheat grain in Canada, produced the type-B trichothecenes (Wong et al., 1995). In Poland, *F. poae* was determined in 35% of wheat samples, while chemical analyses showed no presence of the type-A trichothecene (T-2 toxins) but just the presence of the type-B trichothecenes (Grabarkiewicz-Szczesna et al., 2001). Contrary to these authors, Kosiak et al. (2003) found out that the same species in Norway was the best producer of the type-A trichothecenes (T-2 and HT-2 toxins). A somewhat lower frequency of *F. poae* on wheat grain (13.5%) was recorded by Muthomi et al. (2006) in Kenya. These authors determined only the presence of T-2 toxin and zearalenone in the same substrate.

According to the mycological studies carried out in Serbia by Bočarov-Stančić et al. (1991), only the type-A trichothecene (T-2 toxin), in the amount of 310—780 ppb, was present in wheat grain, originated from a macro-trail in Klek (Vojvodina). Škrinjar et al. (1996) detected zearaleno-

ne just in one sample of wheat, harvested during 1994. In both cases, not a single determined *Fusarium* spp. belonged to *F. poae*. Dopuđa and Lević (2004) and Stojanović et al. (2005) determined the presence of *F. poae* in 12% and 7% of the samples, respectively, but the authors did not present data on their toxigenicity. The results of Dopuđa and Lević (2004), obtained on the basis of studies performed on the samples of five wheat cultivars harvested during 2002 and 2003 at four locations, show that *F. poae* occurred more intensively in the year (2003) in which the frequency of *F. graminearm* was lower, and vice versa. Similar results were achieved by Schaafsma (1999) who stated that the presence of *F. poae*, *F. sporotrichioides* and *F. avenaceum* on wheat grain in Canada was reduced during the years with more intensive occurrence of *F. graminearum*.

Our previous studies performed on *Fusarium* isolates originated from wheat (harvested during 1982 and 1984), cultured on the natural solid substrate, showed that 22% of the observed *Fusarium* spp. biosynthesised DAS, and even 44% produced T-2 toxin, although not a single one belonged to the species *F. poae* (Bočarov-Stančić et al., 1986).

The potential of *F. poae* to biosynthesise one group of fusariotoxins — the type-A trichothecenes (T-2 toxin and DAS), and zearalenone (ZEA) — was observed under *in vitro* conditions in the present study. In our country according to the literature data, a little attention was paid, to the study of the toxicological profile of this species, considering its distribution and toxicogenic properties.

MATERIAL AND METHODS

Cultures of *Fusarium poae.* Isolates of the fungus *F. poae* were obtained from grain samples of wheat, collected in harvest at 20 locations in 2003, 2005 and 2006. A total of 103 isolates of this species were isolated and determined. Out of these 103, 11 isolates, designated as MRIZP-32, MRIZP-37, MRIZP-664, MRIZP-665, MRIZP-666, MRIZP-833, MRIZP-834, MRIZP-860, MRIZP-879, MRIZP-890 and MRIZP-897, were selected for toxicological studies.

Each sample of 32 wheat kernels (four replicates) was analysed. Eight surface sterilised kernels were placed on each water agar (WA) in the 10-cm Petri dishes, and incubated under indoor conditions for seven days. Resulting colonies were purified by the procedure of obtaining monosporous cultures that were then used for the identification of *Fusarium* species. Monosporous cultures were subcultured on the potato dextrose agar (PDA), carnation sterilised leaf-fragment agar (CLA), and synthetic nutrient agar (SNA). Cultures on the PDA were incubated in the dark at 25 ± 1°C, while cultures on the CLA and SNA were incubated under fluorescent and near ultraviolet light for 12 hours at 25 ± 1°C, and in the dark for 12 hours at 20 ± 1°C. The identification of the obtained species was done after Nelson et al. (1983) and Burgess et al. (1994). The identified isolates were stored on the PDA, CLA and

SNA slants in ampoules at +4°C, until studying of their toxicological potential in the liquid culture.

Control isolates. The following species for which it was previously determined (B o č a r o v - S t a n č i ć, unpublished data) to have the capacity to biosynthesise fusariotoxins five weeks after the cultivation on wet maize grain at 30°C, were chosen as the control isolates: a) *F. graminearum*, the isolate GZ-LES that synthesises ZEA and DON at the concentration of 4,420 ppb and 465,900 ppb, respectively; b) *F. sporotrichioides*, KF-38/1/R, a re-isolate of the original strain that biosynthesises T-2 toxin and DAS at the concentration of 2,400 ppb and 1,600 ppb, respectively.

Medium and conditions for the toxin production. All isolates of *F. poae* and both control isolates were grown in the glucose-peptone-yeast extract (GPY) liquid medium. Also, three isolates of *F. poae* and the control isolates were grown in sucrose-peptone-yeast extract (SPY) liquid medium. The GPY liquid medium (pH 5.8) contains 5% of glucose, 0.1% of peptone and 0.1% of yeast extract. The SPY liquid medium (pH 6.5) contains 5% of sucrose, 0.1% of peptone and 0.1% of yeast extract.

Media (100 ml each) were poured into 500-ml Erlenmeyer flasks, and cultured with five fragments (5 x 5 mm) of the fungus that were grown on potato dextrose agar (PDA) in the Petri dishes at 27°C, for seven days. In order to obtain submersed cultures, after inoculation of the medium the Erlenmeyer flasks were kept on the rotary shaker (180 rounds min^{-1}) at room temperature (21—26°C) for three days. The pH value was measured after the incubation of the isolate.

Determination of fusariotoxins. After the cultivation on the rotary shaker, liquid cultures were filtered. Qualitative and quantitative ZEA determinations in filtrates of mould cultures were carried out by applying the multitoxin thin layer chromatographic method, developed by C v e t n i ć et al. (2005). Crude extracts of the type-A trichothecenes (DAS and T-2 toxin) were obtained by the use of ethyl acetate. Each liquid culture (25 ml) was extracted twice with 15 ml of ethyl acetate. Organic extracts were recovered by filtration through the layer of anhydrous sodium sulphate, combined and evaporated almost to dryness, under the rotary evaporator. Further purification was done by the method of R o m e r et al. (1978). The crude oily extract of trichothecenes was dissolved in the methanol/water (1:1, v/v) extraction solvent, which tends to extract compounds of the polarity of trichothecenes, while it does not extract low polar compounds, such as fats and oils. Afterwards, 30% of aqueous ammonium sulphate was added to remove additional interferences. The further step was selective concentration of the analytes into chloroform, and removal of acidic interferences from chloroform extracts, by washing it with the aqueous potassium hydroxide solution. Thinlayer chromatography was performed according to P e p e l j n j a k and B a b i ć (1991) with toluole/ethyl acetate/formic acid developing solvent (5:4:1, v/v/v). All analyses were done in three repetitions.

RESULTS AND DISCUSSION

After the incubation period, with the exception of one *F. poae* isolate (MRIZP-890), the decreased pH value was determined in both liquid media, especially in the control isolates (Tables 1 and 2).

The results obtained in mycotoxicological studies show that *F. poae*, in contrast to *F. graminearum* (isolate GZ-LES), did not have the potential for the zearalenone biosynthesis (Tables 1 and 2). These results are in accordance with literature data (M a r a s a s et al., 1984). Generally, there is a very small number of authors stating that *F. poae* biosynthesises zearalenone, among others K o c i ć - T a n a c k o v (2004). By re-testing numerous toxicogenic isolates of the *Fusarium* species, M a r a s a s et al. (1984) determined that some results obtained on the production of mycotoxins were incorrect as the identification of fungi was not correct.

Tab. 1 — Yields (μg L^{-1}) of zearalenone and type-A trichothecene (DAS, T-2 toxin) in GPY liquid cultures of 11 *F. poae* isolates originated from wheat and control isolates of *F. graminearum* (No. 12) and *F. sporotrichioides* (No. 13)

No.	Isolate designation	Origin	pH	Fusariotoxin yields (μg L^{-1})		
				ZEA	DAS	T-2
1.	MRIZP-32	Inđija	5.30	n.d.*	n.d.**	n.d.**
2.	MRIZP-37	Erdevik	5.50	n.d.*	n.d.**	240
3.	MRIZP-664	Zemun Polje	5.23	n.d.*	n.d.**	n.d.**
4.	MRIZP-665	Zemun Polje	5.40	n.d.*	n.d.**	n.d.**
5.	MRIZP-666	Zemun Polje	5.41	n.d.*	80	n.d.**
6.	MRIZP-833	Lipnički Šor	5.04	n.d.*	n.d.**	n.d.**
7.	MRIZP-834	Stari Banovci	5.63	n.d.*	n.d.**	n.d.**
8.	MRIZP-860	Sombor	5.48	n.d.*	n.d.**	80
9.	MRIZP-879	Loznica	5.52	n.d.*	n.d.**	n.d.**
10.	MRIZP-890	Pazova	5.87	n.d.*	n.d.**	n.d.**
11.	MRIZP-897	Pirot	5.71	n.d.*	n.d.**	n.d.**
12.	GZ-LES	Leskovac	4.40	37	n.d.**	n.d.**
13.	KF-38/1/R[a]	Poland	4.20	n.d.**	240	4,000

[a] — from barley grains; n.d.* — not detected (F^{-1}); n.d.** — not detected (F^{-1})

Tab. 2 — Yields (μg L^{-1}) of zearalenone and type-A trichothecene (DAS, T-2 toxin) in SPY liquid cultures of three *F. poae* isolates (No. 1—3) originated from wheat and control isolates of *F. graminearum* (No. 4) and *F. sporotrichioides* (No. 5)

No.	Isolate designation	Origin	pH	Fusariotoxin yields (mg L^{-1})		
				ZEA	DAS	T-2
1.	MRIZP-666	Zemun Polje	6.09	n.d.*	n.d.**	80
2.	MRIZP-860	Sombor	5.88	n.d.*	n.d.**	80
3.	MRIZP-897	Pirot	5.84	n.d.*	n.d.**	n.d.**
4.	GZ-LES	Leskovac	4.73	n.d.*	n.d.**	n.d.**
5.	KF-38/1/R[a]	Poland	4.69	n.d.*	n.d.**	160

[a] — from barley seeds; n.d.* — not detected (F^{-1}); n.d.** — not detected (F^{-1})

The presence of DAS was recorded in the isolate MRIZP-666 (*F. poae*) and the control isolate KF-38/1/R (*F. sporotrichioides*) in the glucose-peptone-yeast (GPY) liquid medium at the concentration of 80 μg L^{-1} and 240 μg L^{-1}, respectively. In both cases, the DAS yield was low, close to the limit of detection (LOD) of the applied method. Marasas et al. (1984) concluded that some, but not all isolates in *F. poae*, were able to produce DAS, and the ability could be lost rapidly in culture.

The T-2 toxin was detected in the following three *F. poae* isolates: MRIZP-860 (in both liquid cultures — 80 μg L^{-1}), and MRZIP-37 (240 μg L^{-1}) and MRIZP-666 (80 μg L^{-1}) in the GPY and SPY liquid culture, respectively (Table 1). The control culture KF-38/1/R (*F. sporotrichioides*) in the glucose-peptone-yeast extract (GPY) liquid medium, produced this mycotoxin at the concentration of 4,000 μg L^{-1}. However, these values were significantly lower than those recorded with the original strain of this species, in which the production of the T-2 toxin at the concentration of 150,000 μg L^{-1} had been recorded (Mašić et al., 1997). The obtained results can be explained by the fact that a long-term passaging of microorganism isolates, even fungi isolates, on the artificial media leads to a gradual loss of their biochemical properties. Although *F. sporotrichioides* KF-38/1/R were reisolated (KF-38/1R) from sterile, wet maize grain, it is obvious that their initial potential for production was not completely recovered. On the other hand, a low T-2 toxin yield in the *F. poae* isolate (Table 1 and 2) can not be interpreted in such a way, considering that the majority of the isolates were from 2005, hence they were subcultivated under laboratory conditions only for a short period of time. Thus, these isolates of *F. poae* can be considered as non-toxic.

The gained results show that under such conditions of cultivation in the liquid media, the isolates of *F. poae* from wheat originated in Serbia express low potential for biosynthesis of the type-A trichothecenes. Similar results were obtained with isolates of other potentially toxigenic *Fusarium* spp. (*F. oxysporum* and *F. proliferatum*) also determined on wheat. A fairly weak potential for the production of fusariotoxins was evaluated when the cultivation was performed in the liquid medium: 250—320 μg L^{-1} ZEA, i.e. 320 μg L^{-1} DAS and 160 μg L^{-1} T-2 toxin (Bočarov-Stančić et al., 2003). According to our previous studies, greater amounts of the T-2 toxin in *Fusarium* spp. cultures originated from wheat grain from Serbia were recorded only in *F. sporotrichioides* and *F. culmorum* (Bočarov-Stančić, 1996).

Unlike low toxigenic potential of the isolates of *Fusarium* spp., originated from wheat from Serbia, the information gained in the countries of Northern Europe show that yields, especially those of T-2 toxin, were significantly higher at the cultivation of *Fusarium* spp. under laboratory conditions. In Norway, Torp and Langseth (1999) determined the biosynthesis of the T-2 toxin in all tested isolates at the concentration of 25,000—400,000 μg L^{-1}. A *Fusarium* species resembling *F. poae* (= *F. langsethiae* Torp and Nirenberg) was cultured on the PDA, or in the liquid medium with yeast extract and sucrose. Kroiakova et al. (1989) obtained T-2 toxin yields ranging from 50 to 600,000 ppb, when three isolates of *F. sporotrichioides* v. *poae*, originated from wheat grain, harvested in Moscow region, were *in vitro* cultivated. Hor-

nok and Toth (2001) state that the application of the thinlayer chromatography assay revealed no trichothecene producing strain among *F. poae* isolates originated from Hungary.

Considering the presented results, in order to obtain the final answer to the question on the toxicological profile of the *F. poae* isolates in Serbia, it is necessary to carry out additional studies, not only with new isolates of the coming years, but also under different cultivation conditions, first of all on the sterile natural substrates, such as wheat and maize. Marasas et al. (1984) brought forward examples in which differences in the trichothecene production occurred due to conditions and substrates of the *F. poae* cultivation.

CONCLUSIONS

According to the presented results, it can be concluded that *F. poae* isolates from wheat, in contrast to *F. graminearum*, have no potential for the zearalenone biosynthesis.

In the case of the type-A trichothecenes, the diacetoxyscirpenol, i.e. T-2 toxin production was detected only in one culture of *F. poae*, i.e. 15.38% of the studied isolates, respectively. The potential of *F. poae* from wheat, for the type-A trichothecene production was low, as the concentration of DAS and T-2 toxin did not exceed 80 μg L^{-1} and 240 μg L^{-1}, respectively.

An answer to the question on the toxicological profile of the *F. poae* cannot be made, unless other cultivation conditions are not observed, and unless a greater number of isolates of this species, originated from different harvest years of wheat and other cereals that are hosts of this species, are not studied.

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ТОКСИГЕНИ ПОТЕНЦИЈАЛ ИЗОЛАТА *FUSARIUM POAE* ПОРЕКЛОМ СА ПШЕНИЦЕ

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Резиме

У овом раду је у *in vitro* условима проучена способност изолата *F. poae* за биосинтезу једне групе фузариотоксина — трихотецена типа А (Т-2 токсин и диацетоксисцирпенол-ДАС), као и зеараленона (ЗЕА). Токсиколошки профил ове врсте је код нас недовољно испитан с обзиром на њену заступљеност и токсигена својства преме литературним подацима.

Проучавањима је било обухваћено 11 изолата *F. poae*, пореклом са пшенице из 9 локалитета, углавном са подручја Војводине. Највећи број узорака прикупљен је 2005. године, када су климатски услови погодовали интензивнијој појави фузариоза класа ове пољопривредне културе. За одређивање токсиколошког потенцијала *F. poae*, културе одабраних изолата су гајене у течним подлогама (ГПК и СПК) током 3 дана на собној температури (21—26°C) и на ротационој треси-

лици (180 обртаја мин$^{-1}$). Сирови токсини су изоловани из филтрата течних култура испитаних изолата помоћу етил ацетата, док је квантификација микотоксина извршена методом танкослојне хроматографије. Течна култура изолата ГЗ-ЛЕС (*F. graminearum*) је коришћена као контролна култура за утврђивање потенцијала за биосинтезу зеараленона, а КФ-38/1/Р (*F. sporotrichioides*) за оба трихотецена типа А (Т-2 токсин и ДАС).

Добијени резултати показују да *F. poae*, за разлику од *F. graminearum*, не поседује потенцијал за биосинтезу зеараленона. Присуство ДАС-а је утврђено само код једне културе *F. poae* (МРИЗП-666) и контролног изолата *F. sporotrichioides* (КФ-38/1/Р) који су гајени у течном ГПК медијуму. Т-2 токсин је детектован код изолата МРИЗП-666 при гајењу у обема подлогама, као и изолата МРИЗП-37 у ГПК, односно МРИЗП-860 у СПК медијуму. Контролна култура КФ-38/1/Р (*F. sporotrichioides*) производила је Т-2 токсин у концентрацији од 4000 μg L^{-1}.

На основу изнетих података може се закључити да је потенцијал *F. poae* за биосинтезу трихотецена типа А био низак у датим условима с обзиром да концентрација ДАС-а није прелазила 80 μg L^{-1}, односно Т-2 токсина 240 μg L^{-1}.

Имајући у виду приказане резултате, сматрамо да је за добијање коначног одговора на питање о токсиколошком профилу изолата *F. poae* у Србији неопходно предузети додатна испитивања, не само са новим изолатима из година које следе, него и у другим условима култивисања, у првом реду на стерилном природном супстрату као што су пшеница и кукуруз.