UDC 575:633
DOI: 10.2298/GENSR1303749K
Original scientific paper

MATING POPULATIONS OF *Gibberella fujikuroi* (Sawada) S. ITO SPECIES COMPLEX ISOLATING FROM MAIZE, SORGHUM AND WHEAT IN SERBIA

Tamara KOVAČEVIĆ[1], Jelena LEVIĆ[1], Slavica STANKOVIĆ[1], Jelena VUKOJEVIĆ[2]

[1]Maize Research Institute, Zemun Polje, Belgrade-Zemun, Republic of Serbia
[2] University of Belgrade, Faculty of Biology, Belgrade, Republic of Serbia

Kovačević T., J. Lević, S. Stanković, and J. Vukojević (2013): *Mating populations of Gibberella fujikuroi* (Sawada) S. Ito species *complex isolating from maize, sorghum and wheat in Serbia.* Genetika, Vol 45, No. 3, 749-760.

The status of fertility and distribution of mating populations in the *G. fujikuroi* species complex, isolating from maize, sorghum and wheat cultivated under various agroecological conditions of Serbia, have been studied. A total of 79 field isolates of *Fusarium* spp. in the section *Liseola*, which had been reciprocally crossed to standard testers (*MAT-1* and *MAT-2*) from each of the four mating populations of the *G. fujikuroi* species complex, were selected for these studies. Twenty of 79 isolates belonged to the mating population A (*G. moniliformis*), 22 to the population D (*G. intermedia*), 17 to the population E (*G. subglutinans*) and 20 to the population F (*G. thapsina*). A mating type *MAT-1* was dominant in the populations A (14 *MATA-1* : 6 *MATA-2*), D (13 *MATA-1* : 9 *MATA-2*) and E (10 *MATA-1* : 7 *MATA-2*), while *MAT-2* prevailed in the population F (6 *MATA-1* : 14 *MATA-2*). The obtained results indicate that the possibility of sexual reproduction of *Fusarium* spp., belonging to the A, D, E and F mating populations, is not so frequent phenomenon in Serbia as in other regions world-wide. Consequently, these species will be asexually reproduced under field conditions, particularly species belonging to the F population. These are the first results on the characterisation of three (A, E and F) out of four populations of the *G. fujikuroi* species complex present in Serbia.

Key words: *Gibberella fujikuroi*, A, E, D and F populations, mating type, cereals

INTRODUCTION

Gibberella fujikuroi (Sawada) S. Ito is a teleomorph for many species of anamorphs of *Fusarium* section *Liseola*. This species complex includes at least 11 different reproductively

Corresponding author: Jelena Lević, Maize Research Institute, Zemun Polje, Slobodana Bajića 1, 11 185 Belgrade, Republic of Serbia, Fax number: +381 11 37 56 707, E-mail address: *jlevic@mrizp.rs*

isolated biological species or mating populations (MPs) denoted by letters A through K: MP-A (*G. moniliformis*, anamorph *F. verticillioides*), MP-B (*G. sacchari*, anamorph *F. sacchari*), MP-C (*G. fujikuroi*, anamorph *F. fujikuroi*), MP-D (*G. intermedia*, anamorph *F. proliferatum*), MP-E (*G. subglutinans*, anamorph *F. subglutinans*), MP-F (*G. thapsina*, anamorph *F. thapsinum*), MP-G (*G. nygamai*, anamorph *F. nygamai*), MP-H (*G. circinata*, anamorph *F. circinatum*), MP-I (*G. konza*, anamorph *F. konzum*), MP-J (*G. gaditjirrii*, anamorph *F. gaditjirrii*) and MP-K (*G. xylarioides*, anamorph *F. xylarioides*) (MORETTI, 2009).

In Serbia, four *Fusarium* species in the section *Liseola* were identified in numerous plant species including small and millet grains, industrial, medicinal and ornamental plants, weeds and others. *F. verticillioids* was identified in over 30 plants, *F. proliferatum* in 19, *F. suglutinans* in 11 and *F. thapsinum* in one plant species (LEVIĆ, 2008). Species *F. verticillioides* and *F. subglutinans* dominated on maize (LEVIĆ *et al*., 1997, 2009, 2012a), *F. proliferatum* on wheat (LEVIĆ *et al.,* 2012b; STANKOVIĆ *et al*., 2007b) and barley (LEVIĆ et al., 2012b), while *F. thapsinum* dominated on sorghum (LEVIĆ et al., 2009). The frequency of *F. subglutinans* and *F. verticillioides* has changed in the last 15 years. These changes were pronounced in reduced incidence of *F. subglutinans* and increased incidence of *F. verticillioides* (LEVIĆ et al., 1997, 2012a). *Fusarium* species can cause stand reduction, poor grain quality (LEVIĆ, 2000; DESJARDINS *et al.,* 2006; KRNJAJA *et al.,* 2011) and is a potential threat to human and animal health, as some isolates belonging to these species produce fumonisin (LEVIĆ *et al.*, 2004; STANKOVIĆ *et al*., 2008, 2012; TANČIĆ *et al.,* 2012). Despite of the agricultural impacts on *Fusarium* species in Serbia, there is a lack of information on the genetic characterisation of these species on the basis of fertility and mating types (STANKOVIĆ *et al.,* 2007a, b; KRNJAJA *et al.,* 2012).

The goal of this study was to assess the fertility status and the mating type distribution of *Fusarium* species belonging to the section *Liseola* by crossing the field isolates to standard testers of the *G. fujikuroi* species complex.

MATERIALS AND METHODS

Fungal isolates

Seventy nine monospore cultures of *Fusarium* spp. in the section *Liseola* were tested for these studies. Of these 79 isolates, 22, 48 and 9 isolated from grains of maize (*Zea mays* L.), sorghum (*Sorghum bicolor* (L.) Moench.) and wheat (*Triticum aestivum* L.), respectively, collected in the period from 1993 to 2004. Two standard tester strains, *MAT*-1 and *MAT-2*, from each of the four mating populations were used to determine a mating type and female fertility of selected isolates. Standard tester strains were from Italy (Institute of Science of Food Products CNR, Bari): *MATA-1 (1)* (3621), *MATA-2 (1)* (3622), *MATD-1(1)* (3628), *MATD-2 (1)* (3627), *MATE-1 (1)* (3629), *MATE-2 (1)* (3630), *MATF-1 (1)* (3632) and *MATF-2 (1)* (3631), and from Australia (Fusarium Research Laboratory, Department of Crop Science, University of Sydney): *MATA-1(2)* (Q 27), *MATA-2(2)* (Q 26), *MATD-1(2)* (Q 15), *MATD-2(2)* (Q 14), *MATE-1(2)* (Q 23), *MATE-2(2)* (Q 22), *MATF-1(2)* (Q 25) and *MATF-2(2)* (Q 24).

Crosses

A modified mating population (MP) technique (KLITTICH and LESLIE, 1988) was used to determine the affiliation of selected isolates with a mating population of the *G. fujikuroi* species complex. Standard testers, used as female parents (♀), were incubated on carrot agar (CA) in Petri dishes at 20ºC in the dark for 7 days. Selected field isolates, used as male parents

(♂), were incubated on the carnation leaf agar (CLA) slant in a test tube, instead of culturing on a complete medium (CM) recommended by KLITTICH and LESLIE (1988). Cultures of male parents were maintained under the alternating fluorescent and near ultra violet (NUV) light and with a 12-h-12-h day-night cycle. CLA was prepared by using the laboratory manual of BURGESS *et al*. (1994).

Three to five ml of sterile distilled water were added to the test tube with the developed culture of male parent isolates. To obtain a dense suspension, a mycelium and spores were separated from the medium by a spatula. A spore suspension of a male parent was poured over a tester colony (♀) developed in the Petri dish and was carefully distributed by the spatula over the entire surface. Crossed cultures were grown under the alternating fluorescent and NUV light and with a 12-h-12-h day-night cycle for 4 to 6 weeks.

Reciprocal crosses, a tester as a male parent and an isolate as a female parent, were also performed. The procedure was identical as in the fertility determination of the male parent or the tested isolate. If in both cases an isolate formed perithecia or a fertile progeny interspecies cross was performed, that is, a same isolate was used for both female and male parents.

After two weeks, at weekly intervals, cultures of crossed testers and isolates were examined under the stereomicroscope to determine the fertility of progenies. The average biometric values were obtained after 10 perithecia, 30 asci and 30 ascospores of each isolate were measured in two replicates. Total of 1600 perithecia, 4800 asci and 4800 ascospores were measured.

Data analysis

The concept of the effective population size was applied in studies of population genetics of species of the genus *Fusarium*. The effective population size [N_e] in the *G. fujikuroi* species complex was determined on the basis of: (a) number of strains of different mating types [$N_{e(mt)}$], expressed as a percentage of the actual count, and (b) number of male and hermaphrodite strains [$N_{e(f)}$], expressed as a percentage of the actual count (LESLIE and KLEIN, 1996; BRITZ *et al.,* 1998). The effective population size based on the frequency of mating types was determined by the following equation: $[N_{e(mt)}] = (4\ N_m N_f) / N_m + N_f$, where N_m = the total number of isolates (strains) with one mating type (*MAT-1*); N_f = the total number of isolates (strains) with another mating type (*MAT-2*) within the same population. The effective population number (size) [$N_{e(f)}$], based on the relative frequency of female-sterile and hermaphrodite strains, was determined by the following equation: $[N_{e(f)}] = 4N^2 \cdot N_h / (N + N_h)^2$, where N = the total number of female-sterile strains, N_h = the total number of hermaphrodite strains. Variance of the effective population size was estimated by the following equation: $[N_{e(v)}] = N^2 / (N + N_h)$, where N = the total number of female-sterile strains, N_h = the total number of hermaphrodite strains.

RESULTS

Crosses

The progeny was fertile when ascospore-oozing perithecia were observed. The progeny was not considered fertile when ascospores oozed out of only 1-3 perithecia or if small perithecia mostly covered with mycelium were formed.

Of 22 isolates originating from Serbian maize, eight were assigned to A, six to D and eight to E mating population of *G. fujikuroi* species complex (Table 1, 2). Out of 10 isolates from wheat, seven were assigned to D and two to E mating population of this species. Furthermore, out of 48 isolates originating from sorghum, 12 were assigned to A, nine to D, seven to E and 20 to F mating population.

Table 1. Distribution of mating types A and D among the Fusarium isolates section Liseola obtained from maize (Z. mays), sorghum (S. bicolor) and wheat (T. aestivum) in Serbia

Isolates	Sources			Mating type
	Plant species	Site	Year	
74	*Zea mays*	Zemun Polje	1999	*A-1(1)*
97	*Zea mays*	Zemun Polje	2000	*A-1(2)*
106	*Zea mays*	Zemun Polje	1993	*A-1(1)*
121	*Zea mays*	Zemun Polje	1999	*A-1(1)(h)*
572	*Zea mays*	Krnješevci	1998	*A-1(1)*
336	*Sorghum bicolor*	Bački Petrovac	2004	*A-1(1)*
338	*Sorghum bicolor*	Bački Petrovac	2004	*A-1(1)*
343	*Sorghum bicolor*	Bački Petrovac	2004	*A-1(1)*
354	*Sorghum bicolor*	Bački Petrovac	2004	*A-1(1)*
455	*Sorghum bicolor*	Bački Petrovac	2004	*A-1(1)*
485	*Sorghum bicolor*	Bački Petrovac	2004	*A-1(1)*
488	*Sorghum bicolor*	Bački Petrovac	2004	*A-1(1)*
489	*Sorghum bicolor*	Bački Petrovac	2004	*A-1(1)*
22	*Zea mays*	Zemun Polje	2000	*A-2(1)*
557	*Zea mays*	Zemun Polje	1997	*A-2(2)*
574	*Zea mays*	Vršac	1999	*A-2(2)*
405	*Sorghum bicolor*	Bački Petrovac	2004	*A-2(1)*
413	*Sorghum bicolor*	Bački Petrovac	2004	*A-2(2)*
479	*Sorghum bicolor*	Bački Petrovac	2004	*A-2(2)*
506	*Sorghum bicolor*	Bački Petrovac	2004	*A-2(2)*
64	*Zea mays*	Velika Plana	1999	*D-1(1)*
86	*Zea mays*	Inđija	2000	*D-1(1)*
102	*Zea mays*	Zemun Polje	1999	*D-1(1)*
115	*Zea mays*	Krnješevci	1999	*D-1(1)*
567	*Zea mays*	Zemun Polje	2002	*D-1(1)*
212	*Triticum aestivum*	Erdevik	2003	*D-1(1)*
223	*Triticum aestivum*	Sremska Mitrovica	2003	*D-1(1)*
234	*Triticum aestivum*	Sremska Mitrovica	2003	*D-1(1)*
262	*Triticum aestivum*	Bečej	2004	*D-1(1)*
307	*Triticum aestivum*	Bečej	2004	*D-1(1)*
447	*Sorghum bicolor*	Bački Petrovac	2004	*D-1(1)*
467	*Sorghum bicolor*	Bački Petrovac	2004	*D-1(1)*
512	*Sorghum bicolor*	Bački Petrovac	2004	*D-1(1)*
46	*Zea mays*	Zemun Polje	1995	*D-2(1)*
342	*Sorghum bicolor*	Bački Petrovac	2004	*D-2(1)*
348	*Sorghum bicolor*	Bački Petrovac	2004	*D-2(1)*
349	*Sorghum bicolor*	Bački Petrovac	2004	*D-2(1)*
350	*Sorghum bicolor*	Bački Petrovac	2004	*D-2(1)*
460	*Sorghum bicolor*	Bački Petrovac	2004	*D-2(1)*
441	*Sorghum bicolor*	Bački Petrovac	2004	*D-2(1)(h)*
205	*Triticum aestivum*	Inđija	2003	*D-2(1)*
554	*Triticum aestivum*	Sremska Mitrovica	2002	*D-2(1)*

(1) – testers originating from the Institute of Science of Food Products CNR, Bari, Italy

(2) – testers originating from the Fusarium Research Laboratory, Department of Crop Science, University of Sydney, Australia

(h) – hermaphrodite

Table 2. Distribution of mating types E and F among the Fusarium isolates section Liseola obtained from (Z. mays), sorghum (S. bicolor) and wheat (T. aestivum) in Serbia

35	*Zea mays*	Zemun Polje	2000	*E-1(2)*
559	*Zea mays*	Bajmok	2002	*E-1(2)*
560	*Zea mays*	Srbobran	1999	*E-1(2)*
562	*Zea mays*	Zemun Polje	1999	*E-1(2)*
565	*Zea mays*	Zemun Polje	2002	*E-1(2)*
571	*Zea mays*	Zemun Polje	2000	*E-1(2)*
418	*Sorghum bicolor*	Bački Petrovac	2004	*E-1(2)*
424	*Sorghum bicolor*	Bački Petrovac	2004	*E-1(2)(h)*
566	*Sorghum bicolor*	Bački Petrovac	2002	*E-1(2)*
576	*Triticum aestivum*	Zemun Polje	2000	*E-1(2)*
563	*Zea mays*	Zemun Polje	1999	*E-2(2)*
564	*Zea mays*	Zemun Polje	1999	*E-2(2)*
403	*Sorghum bicolor*	Bački Petrovac	2004	*E-2(2)*
432	*Sorghum bicolor*	Bački Petrovac	2004	*E-2(2)*
436	*Sorghum bicolor*	Bački Petrovac	2004	*E-2(2)*
552	*Sorghum bicolor*	Bački Petrovac	2004	*E-2(2)*
21	*Triticum aestivum*	Ruma	2003	*E-2(2)*
376	*Sorghum bicolor*	Bački Petrovac	2004	*F-1(1)*
388	*Sorghum bicolor*	Bački Petrovac	2004	*F-1(1)*
396	*Sorghum bicolor*	Bački Petrovac	2004	*F-1(1)*
463	*Sorghum bicolor*	Bački Petrovac	2004	*F-1(1)*
411	*Sorghum bicolor*	Bački Petrovac	2004	*F-1(2)*
428	*Sorghum bicolor*	Bački Petrovac	2004	*F-1(2)*
323	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*
363	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*
364	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*
379	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*
381	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*
384	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*
385	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*
386	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*
398	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*
422	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*
437	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*
497	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*
529	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*
532	*Sorghum bicolor*	Bački Petrovac	2004	*F-2(2)*

(1) – testers originating from the Institute of Science of Food Products CNR, Bari, Italy
(2) – testers originating from the Fusarium Research Laboratory, Department of Crop Science, University of Sydney, Australia
(h) – hermaphrodite

Of 20 field isolates originating from grain of maize (eight isolates) and sorghum (12 isolates) belonging to the population A of *G. fujikuroi*, the majority successfully produced fertile progenies with testers originating from Italy, and only six isolates with testers originating from Australia (Table 1, 2). Among 20 isolates mating types *MATA-1* and *MATA-2* segregated in the 14:6 ratio, resulting in the reduction of $N_{e(mt)}$ to 84.0% of the actual count (total population) (Table 3). One isolate was hermaphrodite (Table 1) resulting in $N_{e(f)}$ of 18.14% of the actual count (Table 3). All isolates were female-sterile and male-fertile, except the isolate MRIZP 121, isolating from maize, which was hermaphrodite. Based on self-crossing it was determined that this isolate was heterothallic.

Table 3. Experimental data and the parameters of the size of some populations of Gibberella fujikuroi species complex collected from maize, sorghum and wheat in Serbia

Biological species	Mating ratio (*MAT-1* : *MAT-2*)	Effective population number, N_e		
		Mating type, $N_{e(mt)}$	Male/hermaphrodite polymorphism, $N_{e(f)}$	$N_{e(v)}$
A	14:6	84.00	18.14	95.24
D	13:9	96.69	16.64	95.65
E	10:7	96.89	20.84	88.58
F	6:14	84.00	0.00	100.00

$N_{e(f)}$ – number of hermaphrodite isolates

Field isolates originating from grain of maize (six isolates), sorghum (nine isolates) and wheat (seven isolates), belonging to the population D formed mature perithecia with testers originating from Italy, but not with testers for this population originating from Australia (Table 1). Within this population, the *MATD-1* to *MATD-2* ratio was 13:9, resulting in the reduction of $N_{e(mt)}$ to 96.69% of the actual count. Among 22 fertile isolates of the population D, one isolate was hermaphrodite resulting in $N_{e(f)}$ of 16.60% of the actual count (Table 3). It was confirmed by reciprocal crossing that all isolates were female-sterile and male-fertile, apart from the isolate MRIZP 441, which produced fertile progeny either as a female or a male parent with the tester *MATD-1* (3628). This isolate produced sterile progeny in self-crossing.

Seventeen field isolates, originating from grain of maize (eight isolates), sorghum (seven isolates) and wheat (two isolates) that belonged to the population E, produced fertile progeny only with testers originating from Australia and none with testers from Italy (Table 2). Among 17 isolates of the population E, mating types *MATE-1* and *MATE-2* segregated in the 10:7 ratio, resulting in the reduction of $N_{e(mt)}$ to 96.89% of the actual count (Table 3). Reciprocal crosses showed that 16 isolates were female-sterile and male-fertile, while one isolate (MRIZP 424), originating from sorghum, was hermaphrodite. The determination of hermaphrodites resulted in $N_{e(f)}$ of 20.84% of the actual count.

Of 20 isolates, originating from sorghum grain, the majority (16 isolates) produced fertile progeny in crosses with testers of the population F, originating from Australia, while only four isolates formed mature perithecia in crosses with mating types of this population originating from Italy (Table 2). A great difference in segregation of mating types *MATE-1* and *MATE-2* (6:14) resulted in the reduction of $N_{e(mt)}$ to 84.00% of the actual count (Table 3). Reciprocal crosses showed that all tested filed isolates were female-sterile and male-fertile. Fertile progenies were produced in both isolates with yellow pigment and isolates without this pigment in the PDA culture, but with culture properties similar to those produced by isolates of the population A.

Biometric values of mating population

Fertile progenies of all four populations (A, D, F and E) formed round or ovoid blue-black perithecia on the substrate surface either individually or in groups of a few. Asci were pellucid, ellipsoidal or clavate, mainly with 8 and rarely with 4-6 hyaline, mostly 1- and sometimes 2-3-septate ascospores, which were straight gently tapering towards septa.

On the whole, smaller perithecia were produced by isolates of the population F or *G. thapsina*, while the largest perithecia with the greatest variation were produced by isolates of the population D or *G. intermedia* (Table 4). Biometric values of perithecia for the population E or *G. subglutinans* were somewhat greater than the values of perithecia for the population A or *G. moniliformis*. The ascus sizes were approximately equal for populations A, D and F, while the greatest departure from them was shown by the population E. The smallest ascospores were formed by isolates that belonged to the population D, while almost the same values were observed in populations A, F and E.

Table 4. Biometric values of perithecia, asci and ascospores of different mating populations (MP) or G. fujikuroi species complex formed in crosses of MRIZP isolates to standard testers

Value	Dimensions (µm)					
	Perithecia		Asci		Ascospores	
	Height	Width	Length	Width	Length	Width
	MP-A (*G. moniliformis*)					
Min	240	210	56.1	1.66	9.9	3.3
Max	495	405	89.1	12.2	19.8	9.9
X ± d	332.5 ± 33.50	290.32 ± 8.79	67.44 ± 7.29	8.98 ± 1.59	15.12 ± 2.86	5.79 ± 1.48
	MP-D (*G. intermedia*)					
Min	255	225	56.1	6.6	9.9	3.3
Max	540	405	85.8	13.2	19.8	9.9
X ± d	392.3 ± 45.45	318.2 ± 35.92	68.01 ± 7.0	8.24 ± 1.59	12.98 ± 2.11	5.47 ± 1.61
	MP-E (*G. subglutinans*)					
Min	270	240	56.1	6.6	9.9	3.3
Max	450	390	99	13.2	19.8	9.9
X ± d	390.42 ± 30.95	352.5 ± 26.2	75.25 ± 7.24	9.31 ± 1.32	14.98 ± 2.69	6.14 ± 1.31
	MP-F (*G. thapsina*)					
Min	270	240	52.8	6.6	9.9	3.3
Max	480	405	92.4	9.9	23.1	6.6
X ± d	358.3 ± 25.42	279.4 ± 27.67	69.89 ± 8.24	8.71 ± 1.96	15.42 ± 3.20	6.40 ± 1.03

DISCUSSION

The application of the MP technique showed that populations A, D, E, and F in the *G. fujikuroi* species complex existed in Serbia. The population A isolated from both, maize and sorghum, the populations D and E isolated from maize, sorghum and wheat, while the population F isolated only from sorghum.

The domination of a mating type depends on environments, ecological niche within which each biological species develops, hosts and a year (LESLIE, 1995; KIM *et al.,* 2012). In

nature, one mating type and female-sterile but male-fertile individuals within a certain population of the *G. fujikuroi* species complex dominate. Less common are cases that mating types segregate in the equal ratio that follows a Mendelian ratio of 1:1 (CHULZE *et al*., 2000; CUMAGUN, 2007).

This study showed the domination of mating type *MAT-1* in populations A, D and E isolating from maize and A isolating from sorghum in Serbia. On other hand, *MAT-2* dominated in populations D, E and F isolating from sorghum. Similar results for populations A, D, and F isolating from sorghum were obtained in Korea (LIM *et al.,* 2001) and for the population A isolating from sorghum in Brazil (SILVA *et al.,* 2006). Domination of *MAT-1* isolating from maize was found in Egypt (SABET *et al.,* 2006) and Iran (MOHAMMADIAN *et al.,* 2011). Contrarily, *MAT-2* was more frequency than *MAT-1* isolating from maize in Brazil (SILVA *et al.,* 2006).

Variation of distribution of one mating type has been determined within one country (CHULZE *et al.,* 2000; REYNOSO *et al.,* 2006). Data obtained in the present study point out to the dominance of *MAT-1* in the population D isolating from maize. But, data presented by other Serbian authors indicate the dominance of *MAT-2* in this population isolating from maize (STANKOVIĆ *et al*., 2007a; KRNJAJA *et al.,* 2012) and onion (STANKOVIĆ *et al.,* 2007c). In Korea, KIM *et al*. (2012) gained different results on the frequency of a mating type pair in the populations A, D and E over years and hosts.

Of 20 isolates in the population A only one hermaphrodite was identified, which resulted in $N_{e(f)}$ (male/hermaphrodite polymorphism) of 18.14% of the actual count. Contrary to this, DANIELSEN *et al*. (1998) in Costa Rica found that this parameter amounted to 98% due to a higher frequency of hermaphrodites. REYNOSO *et al.* (2006) in Argentina and SABET *et al.* (2006) in Egypt identified a greater number of hermaphrodites not only in the population A, but also in the population D.

Unlike LIM *et al.* (2001), who estimated a relatively high frequency of hermaphrodites in the population F, especially in the mating type *MAT-1*, we did not identify any hermaphrodite in our study. Therefore, $N_{e(f)}$ and $N_{e(v)}$ in the population F were 0 and 100% of the actual count, respectively. According to BRITZ *et al.* (1998) if the population is relatively recent and has not yet reached equilibrium, then it is likely that the number of hermaphrodites would continue to fall and if eliminated completely from the population could lead to a totally asexual population and an evolutionary dead end. It was similar in our study, because the population F was identified in Serbia for the first time in 2004 (LEVIĆ *et al*., 2006). Furthermore, LESLIE and KLEIN (1996) point out that the relative rarity of sexual reproduction may permit female-sterile strains to accumulate to a level such that local populations could form new species or subspecies as a result of their isolation. BRITZ *et al.* (1998) considered that monitoring the population by sampling it on a yearly basis should be replaced by the analysis of samples recovered over a period of 10 years or more.

In nature, only the population A (anamorph *F. verticillioides*) of four populations (A, D, E and F) forms perithecia (SUMMERELL *et al.,* 1998). However, in Serbia such possibilities are limited, because there is only one hermaphrodite that functions as both parents, female and male, while the remaining members of this population are female-sterile and male-fertile strains. Obtained data indicate that in Serbia asexual reproduction will prevail in all four species that belong to the populations A, D, E or F of *G. fujikuroi* species complex.

Gained results on biometric values of anamorph of *Gibberella* point out to small differences in appearance of fruiting bodies, asci and ascopsores among four tested species. Perithecia are black on the medium, while they are bluish on the native preparation. Peridia are

scabrous or warty. Biometric values also point out to small differences. The appearance, structure and values of perithecia, asci and ascospore of isolates that were identified as *G. moniliformis*, *G. intermedia* and *G. subglutinans* are in accordance with the data presented for the same species by KUHLMAN (1982). There are certain differences as KUHLMAN (1982) states that the first two species formed 4-septate ascospores, as well as that 3-septate ascospores prevailed in these species. Although obtained biometric values of the *G. thapsina* isolates significantly varied in comparison with results on the same species published by KLITTICH *et al*. (1997), the average values were very similar.

The use of two different sources of standard testers for one mating type provided various results regarding progeny fertility in crosses with field isolates originating from Serbia. Isolates assigned to the population D produced fertile progeny only with the standard tester pair (*MATE-1* and *MATE-2*) from Italy, while population E isolates produced fertile progeny only with the standard tester pair from Australia. The isolates from other two mating populations (A and F) produced fertile progeny with one standard tester pair (from Italy) or with another standard tester pair (from Australia). These differences may be the result of the existence of cryptic species within morphologically similar isolates. STEENKAMP *et al.* (2002) revealed the presence of two major groups representing cryptic species in *F. subglutinans*. Described Group 1 of *F. subglutinans* corresponds to the *F. temperatum*, and the *F. subglutinans* strictly circumscribes the second group, namely *F. subglutinans* Group 2 (SCAUFLAIRE *et al.,* 2012).

In conclusion, the MP technique was found to be useful in assisting the correct identification of *Fusarium* species section *Liseola*, especially of *F. thapsinum*, species morphologically closely related to *F. verticillioides*. Furthermore, obtained results indicate that the mating populations of *G. fujikuroi* present in Serbia (A, D, E and F) show a low potential of genetic recombination in the field. Future research should be undertaken to elucidate the possible presence of cryptic species when standard tester isolates are used for the same mating type and mating population but of geographically diverse backgrounds. In addition, these testers and field isolates should be studied at the molecular level.

ACKNOWLEDGEMENT

The study is a part of the research performed within the scope of the Project No. TR-31023 financially supported by the Ministry of Education and Science of the Republic of Serbia.

. Received August 03th, 2013
Accepted October 05th, 2013

REFERENCES

BRITZ, H., M.J. WINGFIELD, T.A. COUTHINO, W.F.O. MARASAS, J.F. LESLIE (1998): Female fertility and mating type distribution in a South African population of *Fusarium subglutinans* f. sp. *pini*. Appl. Environ. Microbiol., *64 (4)*, 2094-2095. 13.

BURGESS, L.W., B.A. SUMMERELL, S. BULLOCK, K.P. GOTT, D. BACKHOUSE (1994): Laboratory Manual for *Fusarium* Research. Third Edition. *Fusarium* Research Laboratory, Department of Crop Science, University of Sydney and Royal Botanic Gardens, Sydney, 1-133.

CHULZE, S. N., M. L. RAMIREZ, A. TORRES, J. F. LESLIE (2000): Genetic variation in *Fusarium* Section *Liseola* from no-till maize in Argentina. Appl. Environ. Microbiol., *66 (12)*, 5312–5315.

CUMAGUN, C.J.R. (2007): Female fertility and mating type distribution in a Philippine population of *Fusarium verticillioides*. J. Appl. Genet., *48 (4)*, 123–126.

DANIELSEN, S., U.M. MEYER, D.F. JENSEN (1998): Genetic characteristics of *Fusarium verticillioides* isolates from maize in Costa Rica. Plant Pathol., *47*, 615-622.

DESJARDINS, A. E., M. BUSMAN, R. PROCTOR, & R. J. STESSMAN (2006). Wheat kernel black point and fumonisin contamination by *Fusarium proliferatum*. Food Add. Cont., 24, 1131–1137.

KIM, J-H., M-R. KANG, H-K. KIM, S-H. LEE, T. LEE, S-H. YUN (2012): Population structure of the *Gibberella fujikuroi* species complex associated with rice and corn in Korea. Plant Pathology J., *28 (4)*, 357-363.

KLITTICH, C.J.R., P.E. NELSON, J.F. LESLIE, W.F.O. MARASAS (1997): *Fusarium thapsinum* (*Gibberella thapsina*): A new species in section *Liseola* from sorghum. Mycologia, *89 (4)*, 643-652.

KLITTICH, C.R.J., J.F. LESLIE (1988): Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics, *118*, 417-423.

KRNJAJA, V., J. LEVIĆ, S. STANKOVIĆ, A. STEPANIĆ (2011): *Fusarium* species and their mycotoxins in wheat grain. Proc. Nat. Sci. Matica Srpska Novi Sad, *120*, 41-48.

KRNJAJA, V., J. LEVIĆ, S. STANKOVIĆ, S. TANČIĆ (2012): Genetic, pathogenic and toxigenic variability by *F. proliferatum* isolated from maize kernels. Afr. J. Biotechnol., *11 (20)*, 4660-4665.

KUHLMAN, E.G. (1982): Varieties of *Gibberella fujikuroi* with anamorphs in *Fusarium* section *Liseola*. Mycologia, *74 (5)*, 759-768.

LESLIE, J.F. (1995): *Gibberella fujikuroi:* Available populations and variable traits. Can. J. Bot., *73 (Suppl. 1)*, S282 - S291.

LESLIE, J.F., K.K. KLEIN (1996): Female fertility and mating type effects on effective population size and evolution in filamentous fungi. Genetics, *144 (2)*, 557-567.

LEVIĆ, J. (2000): Uticaj *Fusarium moniliforme, F. subglutinans* i *F. proliferatum* na klijavost semena kukuruza. Zbornik izvoda, III jugoslovenski naučno-stručni simpozijum iz selekcije i semenarstva, Zlatibor, 28. maj – 1. jun 2000, 120.

LEVIĆ, J. (2008): Vrste roda *Fusarium* u oblasti poljoprivrede, veterinarske i humane medicine. Institut za kukuruz „Zemun Polje“, Beograd-Zemun, i Društvo genetičara Srbije (ed.), Cicero, Beograd.

LEVIĆ, J., T. KOVAČEVIĆ, S. STANKOVIĆ, D. IVANOVIĆ, J. BERENJI (2006): The first report on *Fusarium thapsinum* on sorghum grains in Serbia. Book of Abstracts, European Fusarium Seminar, Wageningen, The Netherlands, 19-22 September, 2006, 52.

LEVIĆ, J., S. STANKOVIĆ, A. BOČAROV-STANČIĆ, M. ŠKRINJAR, Z. MAŠIĆ (2004): The overview on toxigenic fungi and mycotoxins in Serbia and Montenegro. Pp. 201-218. *In:* A. Logrieco, A. Visconti (eds), An Overview on Toxigenic Fungi and Mycotoxins in Europe, Kluwer Academic Publishers, Dordrecht, Boston, London.

LEVIĆ, J., S. STANKOVIĆ, V. KRNJAJA, A. BOČAROV-STANČIĆ, D. IVANOVIĆ (2012a): Distribution frequency and incidence of seed-borne pathogens of some cereals and industrial crops in Serbia. Pestic. Phytomed. (Belgrade), *27 (1)*, 33–40.

LEVIĆ, T.J., S.Ž. STANKOVIĆ, V.S. KRNJAJA, S.A. BOČAROV-STANČIĆ (2009): *Fusarium* species: The occurrence and the importance in agriculture of Serbia. Proc. Nat. Sci. Matica Srpska Novi Sad, *116*, 33-48.

LEVIĆ, J., S. STANKOVIĆ, V. KRNJAJA, S. TANČIĆ, D. IVANOVIĆ, A. BOČAROV-STANČIĆ *(2012b):* Relationships of mycobita on rachides and kernels of wheat. Eur. J. Plant Pathol., *134 (2)*, 249-256.

LEVIĆ, J., LJ. TAMBURIĆ-ILINČIĆ, T. PETROVIĆ (1997): Maize kernel infection by *Fusarium* species in the period 1994-1996. Cereal Res. Commun., *25 (3)*, 773-775.

LIM, S-H., S-H. YUN, Y-W. LEE (2001): Mating behavior, mycotoxin production, and vegetative compatibility of *Gibberella fujikuroi* species complex from sorghum in Korea. Plant Pathology J., *17 (5)*, 276-280.

MOHAMMADIAN, E., M. JAVAN-NIKKHAH, S.M. OKHOVVAT, K. GHAZANFARI (2011): Study on genetic diversity of *Gibberella moniliformis* and *G. intermedia* from corn and rice, and determination of fertility status and of mating type alleles. Aust. J. Crop Sci., *5 (11)*, 1448-1454.

MORETTI, A.N. (2009): Taxonomy of *Fusarium* genus, a continuous fight between lumpers and splitters. Proc. Nat. Sci. Matica Srpska Novi Sad, *117*, 7-13.

REYNOSO, M.M., A.M. TORRES, S.N. CHULZE (2006): Biological species in the *Gibberella fujikuroi* species complex isolated from maize kernels in Argentina. Plant Pathology J., *5 (3)*, 350-355.

SABET, K.K., A.M.A. ASHOUR, E.M. EL-ASSIUTY, E.M. EL-SHABRAWY (2006): Mating populations and effective population number in *Gibberella fujikuroi* species complex of rotted maize ears under Egyptian conditions. Egyp. J. Phytopathol., *34 (2),* 29-41.

SCAUFLAIRE, J., M. GOURGUE, A. CALLEBAUT, F.MUNAUT (2012): *Fusarium temperatum*, a mycotoxin-producing pathogen of maize. Eur. J. Plant Pathol., *133*, 911–922.

SILVA, V.N., F.M.C. FERNANDES, A. CORTEZ, D.H.B. RIBEIRO, A.P. ALMEIDA, R.H. HASSEGAWA, B. CORRÊA (2006): Characterization and genetic variability of *Fusarium verticillioides* strains isolated from corn and sorghum in Brazil based on fumonisins production, microsatellites, mating type locus, and mating crosses. Can. J. Microbiol., *52 (8)*, 798–804.

STANKOVIĆ, S., J. LEVIĆ, D. IVANOVIĆ, V. KRNJAJA, G. STANKOVIĆ, S. TANČIĆ (2012): Fumonisin B_1 and its co-occurrence with other fusariotoxins in naturally-contaminated wheat grain. Food Control, *23 (2)*, 384-388.

STANKOVIĆ, S., J. LEVIĆ, T. PETROVIĆ, V. KRNJAJA (2008): Toxicological profile of *F. proliferatum* isolated from maize seed, root and stalk. Cereal Res. Commun., *26* (*Suppl. B*), 397-398.

STANKOVIĆ, S., J. LEVIĆ, D. IVANOVIĆ (2007a): Genetic variability of maize pathogens in Serbia. Genetika, *39 (2)*, 227-240.

STANKOVIĆ, S.Ž., J.T. LEVIĆ, V.S. KRNJAJA, S.A. BOČAROV-STANČIĆ, S.L. TANČIĆ, T.M. KOVAČEVIĆ (2007b): Frequency of toxigenic *Fusarium* species and fusariotoxins in wheat grain in Serbia. Proc. Nat. Sci. Matica Srpska Novi Sad, *113*, 93-102.

STANKOVIĆ, S., J. LEVIĆ, T. PETROVIĆ, A. LOGRIECO, A. MORETTI (2007c): Pathogenicity and mycotoxin production by *Fusarium proliferatum* isolated from onion and garlic in Serbia. Eur. J. Plant Pathol., *118 (2)*, 165-172.

STEENKAMP, E. T., B. D. WINGFIELD, A. E. DESJARDINS, W. F. O. MARASAS, M. J. WINGFIELD (2002): Cryptic speciation in *Fusarium subglutinans*. Mycologia, *94 (6)*, 1032-1043.

SUMMERELL, B.A., L.W. BURGESS, S. BULLOCK, D. BACKHOUSE, D.T. NGUYEN (1998): Occurrence of perithecia of *Gibberella fujikuroi* mating population A (*Fusarium moniliforme*) on maize stubble in northern Vietnam. Mycologia, *90 (5)*, 890-895.

TANČIĆ, S., S. STANKOVIC, J. LEVIĆ, V. KRNJAJA, J. VUKOJEVIĆ (2012): Diversity of the *Fusarium verticillioides* and *F. proliferatum* isolates according to their fumonisin B_1 production potential and origin. Genetika, *44 (1)*, 163-176.

POLNE POPULACIJE KOMPLEKSNE VRSTE *Gibberella fujikuroi* (Sawada) S. ITO POREKLOM IZ KUKURUZA, GAJENOG SIRKA I PŠENICE U SRBIJI

Tamara KOVAČEVIĆ[1], Jelena LEVIĆ[1], Slavica STANKOVIĆ[1], Jelena VUKOJEVIĆ[2]

[1]Institut za kukuruz, Zemun Polje, Beograd-Zemun, Republika Srbija
, Univerzitet u Beogradu, Biološki fakultet, Beograd, Republika Srbija

Izvod

U radu su proučavani fertilnost i distribucija polnih populacija i tipova u okviru kompleksne vrste *G. fujikuroi*, poreklom iz različitih biljnih vrsta (kukuruza, gajenog sirka i pšenice), koje su gajene u različitim godinama i agroekološkim uslovima Srbije. Za proučavanja su odabrana 79 poljskih izolata *Fusarium* spp. iz sekcije *Liseola*, koji su recipročno ukršteni sa standardnim testerima (*MAT-1* i *MAT-2*) od četiri polne populacije kompleksne vrste *G. fujikuroi*. U ukrštanjima je fertilno potomstvo dalo 77 od 79 izolata. Od 77 izolata 20 je pripadalo polnoj populaciji A (*G. moniliformis*), 22 populaciji D (*G. intermedia*), 15 populaciji E (*G. subglutinans*) i 20 populaciji F (*G. thapsina*). Polni tip *MAT-1* je dominirao u populacijama A (14 *MATA-1* : 6 *MATA-2*), D (13 *MATA-1* : 9 *MATA-2*) i E (10 *MATA-1* : 7 *MATA-2*), dok je *MAT-2* dominirao u populaciji F (6 *MATA-1* : 14 *MATA-2*). Proučavani izolati su bili fertilni kao očevi a sterilni kao majke, izuzev po jedan izolat u populacijama A, D i E koji su bili hermafroditi. Na osnovu polnog tipa ($N_{e(mt)}$) efektivni broj za A i F populacije bio je 84.00%, za D je 96.69% i E je 96.89%. Na osnovu učestalosti hermafrodita efektivan broj ($N_{e(f)}$) za populaciju A bio je 18.15%, populaciju D 16.60%, populaciju E 20.84% i populaciju F 0.00% od stvarnog broja. Dobijeni rezultati ukazuju da je mogućnost seksualne reprodukcije *Fusarium* spp., koje pripadaju utvrđenim polnim populacija, nije učestala pojava u Srbiji, kao što je za njih utvrđeno u drugim regionima sveta. Kao posledica toga, ove vrste će se aseksualno reprodukovati u poljskim uslovima, posebno vrsta koja pripada F populaciji. Ovo su prvi rezultati o karakterizaciji tri (A, E i F) od četiri populacije kompleksne vrste *G. fujikuroi*, koje su prisutne u Srbiji.

Primljeno 03. VIII 2013.
Odobreno 05. X. 2013.