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PATHOGENICITY AND DIVERSITY OF VEGETATIVE COMPATIBILITY OF *FUSARIUM VERTICILLIOIDES*

ABSTRACT: Pathogenicity of 10 *Fusarium verticillioides* isolates, originated from grain of wheat (five isolates) and maize (five isolates), were studied under greenhouse conditions. Based on different parameters of the pathogenicity estimate (a scale for % of nonemerged plants, % of survived plants, plant vigour — the growth and dry weight of roots and epicotyls and disease severity) it was determined that all *F. verticillioides* isolates expressed a different degree of pathogenicity. According to % of nonemerged plants six, three and one *F. verticillioides* isolates expressed low, moderate and high deegre of pathogenicity, respectively. All *F. verticillioides* isolates reduced the plant survival rate and vigour, while the disease severity ranged from 2.0 to 3.54. Two types of *nit* mutants, *nit1* and NitM, were obtained by the use of the method of vegetative compatibility. The frequency of *nit1* mutants was greater (58.79%) than the frequency of NitM mutants (5.77%). A total of 10 vegetative compatibility groups (VCGs) of *F. verticillioides* were established in the complementation tests. These results point out to a high genetic diversity of *F. verticillioides* population.

KEY WORDS: *Fusarium verticillioides*, pathogenicity, vegetative compatibility

INTRODUCTION

Fusarium verticillioides (Sacc.) Nirenberg (syn. *Fusarium moniliforme* Scheldon) is a widely distributed pathogen of maize (*Zea mays* L.) and many other plant species. In Serbia, *F. verticillioides*, as a pathogen of grain, was identified on maize, wheat and sorghum up to 77.8% (Lević et al., 1997, 2003), 10% (Dopuđa and Lević, 2004) and 7.5% (Lević et al., 2006), respectively.

The epidemiological studies show that *F. verticillioides* has one comparative advantage over other species of the genus *Fusarium*, especially in relation to *F. graminearum* Schwabe, as it requires a greater range of temperatures (Reid et al., 1999) and humidity (Schneider and Pendery 1983) for

its development, hence the competitiveness of the fungus will not change in different environments. It is typical of this species to colonise the plant tissue and to remain at the dormant stage or at the endophytic stage, as long as the tissue is healthy and active (Munkvold and Desjardins, 1997).

It is difficult to discuss with certainty the role of *F. verticillioides* in the etiology of seedling diseases, as seedling infections and the disease development are produced by the seed affected by a disease or a contaminated soil, and depend on the temperature during the maize growing period (Kommedahl and Windels, 1981). *F. verticillioides* does not affect seed germination at the endophytic stage, but it affects the thickness, height, weight and leaf length of seedlings developed from infected seeds (Yates et al., 1997). On the other hand, some strains of this fungus can even stimulate an earlier growth of seedlings.

Lević (2000) established that the frequency of occurrence of *Fusarium* species is not always correlated with their effects on seed germination. According to this author, the following species are most often isolated from maize grain: *F. verticillioides* (50.2%), *F. subglutinans* (Wollenw. & Reinking) Nelson (45.6%) and then *F. proliferatum* (Matsushima) Nirenberg (7.9%). However, germination of seeds infected with *F. subglutinans*, *F. proliferatum* and *F. verticillioides* amounted to 15.3%, 23.4% and 32.6%, respectively.

The characterization of *F. verticillioides* isolates can be done on the basis of the seedling pathogenicity test or vegetative compatibility, since it was determined that isolates of the similar pathogenicity belonged to the same vegetative compatibility group (VCG) (Klein and Correll, 2001). Therefore, if a rapid method of the VCG identification is developed and rapid analyses of the population strain evaluation are provided, then the VCG pathogen strain identification can replace the pathogenicity test, which is time — consuming and requires specific, control led conditions, depending on a plant species.

Considering the economic importance of *F. verticillioides*, pathogenicity of *F. verticillioides* isolates, originating in maize and wheat grown at different locations in Serbia, to maize seedlings and their vegetative compatibility were observed in this study.

MATERIAL AND METHODS

Fungal isolates

Ten isolates of *F. verticillioides* were used to perform pathogenicity and vegetative compatibility tests. Five isolates originated from grain of commercial maize hybrids grown in the vicinity of Belgrade—Zemun, and five isolates originated from grain of wheat varieties grown at different locations in Serbia (Table 1). Isolates were identified as *F. verticillioides* using of the procedure outlined by Nelson et al. (1983) and Burgess et al. (1994).

Tab. 1 — *F. verticilioides* isolates tested for pathogenicity to maize seedlings under greenhouse conditions and vegetative compatibility

No.	Isolate	Origin	Host
1.	MGA-7	Belgrade—Zemun	Commercial maize hybrid
2.	MGD-4	Belgrade—Zemun	Commercial maize hybrid
3.	MGE-5	Belgrade—Zemun	Commercial maize hybrid
4.	MGG-13	Belgrade—Zemun	Commercial maize hybrid
5.	MGI-1	Belgrade—Zemun	Commercial maize hybrid
6.	MRIZP-201	Inđija	Evropa 90 (wheat variety)
7.	MRIZP-237	Inđija	Pobeda (wheat variety)
8.	MRIZP-570	Ruma	Renesansa (wheat variety)
9.	MRIZP-748	Loznica	Simonida (wheat variety)
10.	MRIZP-830	Sombor	Evropa 90 (wheat variety)

Selected cultures were initiated from single conidia and stored on PDA slants at 4°C, until use for the pathogenicity test and vegetative compatibility.

Pathogenicity test with maize seedlings

An insignificantly modified method described by Molot and Simone (1967) was followed for estimations of pathogenicity of *F. verticillioides* isolates. Petri dishes with the two-layer filter paper, instead of flasks, and sterile quartz sand, instead of soil, were used for the development of the fungus and artificial inoculation of seeds. A total of 45 maize seeds, surface-sterilised with sodium hypochlorite per isolate were inoculated in the sterile Petri dishes (ø 100 mm) with 30 ml of spore suspension (2—3 x 10^6 spore ml^{-1}). The spore suspension was prepared from 7—10 old isolates cultured on the PDA at room temperature. Inoculated and non-inoculated (control) maize seeds were incubated at 22°C for two days and at 10°C for three days, and then planted into flats (40 x 18 x 16 cm) with sterile quartz sand, watered and incubated at 24—26°C.

Maize seeds were inoculated for two weeks and the following was determined: degree of pathogenicity, length (cm) and dry weight (g) of seedling roots and epicotyls. In this study, the degree of pathogenicity was defined on the basis of nonemerged plants (%), which was an outcome of seeds that had never germinated, and germinated seeds with completely rotted shoots. According to this parameter, the isolates were classified into five categories based on the scale described by Maçka (1989) (Table 2).

Tab. 2 — The scale for the estimation of pathogenicity of *F. verticillioides* isolates

Percentage of nonemerged plants	Degree of pathogenicity
0—10%	not pathogenic
11—20%	very low pathogenic
21—40%	low pathogenic
41—60%	moderate pathogenic
61—80%	high pathogenic
81—100%	very high pathogenic

Disease severity was also used as a measurement of pathogenicity of isolates, and was rated by a six-class scale, in which 0 = healthy root and epicotyl, and 5 = nongerminated seed, or completely rotted root and shoot. The length of each seedling from the seed attachment site to the top of the longest root and leaves was measured (cm). The detached root and epicotyl per replicate were dried at 60°C for 24 hours and then, their weights (g) were measured. Means were compared by Duncan's multiple range test.

Vegetative compatibility groups

Methods described by Correll et al. (1987) and Kedera et al. (1994) were used to isolate and characterize *nit*-mutants and their mutual pairing in order to determine vegetative compatibility of the studied *F. verticillioides* isolates. The excised pieces of mycelia were planted on the minimum medium (a basal medium amended with 30 g $KClO_3$, 2 g $NaNO_3$, and 1.6 g L-asparagine) for the selection of mutants (sectors). The basal medium contains 1.0 g KH_2PO_4; 0.5 g $MgSO_4$ x $7H_2O$; 0.5 g KCl; 10 mg $FeSO_4$ x $7H_2O$; 0.2 ml sterile solution of microelemenats; 30.0 g of sucrose; 20.0 g of Difco agar; 1000 ml of distilled water. Pieces of hyphae and loose growing sectors were transferred to the basal medium with different nitrogen sources ($NaNO_3$, $NaNO_2$ and hypoxantine) in order to determine the type of the *nit*-mutant on the basis of a phenotype (Puhalla, 1985).

Complementary *nit1* and NitM mutants from each of 10 *F. vertillicioides* isolates were paired on the minimum medium (MM) in all possible combinations to perform complementation tests among the isolates. The *nit* mutants grew very sparsely across the medium, but complementation of auxotrophic mutants was indicated by a line of a vigorous growth where the mutants interacted.

RESULTS AND DISCUSSION

Pathogenicity

All observed *F. verticillioides* isolates affected the survival rate and vigour of plants. Out of 10 *F. verticillioides* isolates tested under greenhouse conditions six, three and one isolates were low (26.67—40.0% of nonemerged plants), moderate (48.87—55.53% of nonemerged plants) and high (62.20% of nonemerged plants) pathogenic (Table 3). The isolate MGG-13, originated from maize grain, was estimated as high pathogenic as it reduced germination by 62.20%. The same isolate was significantly more pathogenic than the remaining isolates, as determined disease severity (3.69) was the highest and the survival rate (37.80%) and plant vigour were the lowest (Table 4).

Tab. 3 — *F. verticillioides* isolates classified on the basis of a percentage of nonemerged plants

Isolate	Nonemerged plants (%)	Degree of pathogenicity
MGA-7	31.13	low pathogenic
MGD-4	40.00	low pathogenic
MGE-5	26.67	low pathogenic
MGG-13	62.20	high pathogenic
MGI-1	55.53	moderate pathogenic
MRIZP-201	48.87	moderate pathogenic
MRIZP-237	37.80	low pathogenic
MRIZP-570	51.13	moderate pathogenic
MRIZP-748	40.00	low pathogenic
MRIZP-830	35.54	low pathogenic
Control	4.47	

The survival rate of seedlings developed from inoculated seeds with different isolates of *F. verticillioides* varied from 37.80% (MGG-13) to 73.33% (MGE-5), which was significantly lower than in the control (95.53%) (Table 4). The observed isolates affected the reduction of the root growth in comparison with the epicotyl growth. The isolate MGI-1, originated from maize, as well as, isolates MRIZP-201 and MRIZP-570, originated from wheat, expressed similar pathogenicity, which was particularly established on the basis of the root growth. The isolate MGD-4 showed peculiar behaviour, as disease severity caused to seedlings was high (3.18), which made it similar to the high pathogenic isolate (MGG-13), but due to a relatively high survival rate of plants (60.00%), it was estimated as low pathogenic (Table 4).

Tab. 4 — Effect of *F. verticillioides* isolates on maize seedlings growing from artificially infected seeds under greenhouse conditions

No.	Isolate	Plant survival* (%)	Plant vigour*				Disease severity*
			Length (cm)		Dry weight (g)		
			Root	Epicotyl	Root	Epicotyl	
1.	MGA-7	68.87^{bc}	18.73^{bcd}	14.71^{bc}	2.10^{bcd}	0.800^{abc}	2.66^{bcd}
2.	MGD-4	60.00^{bcd}	15.87^{cd}	12.14^{bc}	1.37^{cde}	0.500^{bc}	3.18^{abc}
3.	MGE-5	73.33^{ab}	23.74^{b}	16.86^{ab}	2.23^{bc}	0.767^{abc}	2.00^{d}
4.	MGG-13	37.80^{d}	11.42^{d}	8.98^{c}	1.10^{e}	0.400^{bc}	3.69^{a}
5.	MGI-1	44.47^{cd}	13.79^{d}	9.87^{c}	1.33^{de}	0.367^{c}	3.41^{abc}
6.	MRIZP-201	51.13^{bcd}	11.26^{d}	12.15^{bc}	1.00^{e}	0.833^{ab}	3.54^{ab}
7.	MRIZP-237	62.20^{bcd}	23.22^{bc}	18.26^{ab}	2.00^{bcd}	0.833^{ab}	2.54^{bcd}
8.	MRIZP-570	48.87^{bcd}	15.34^{d}	13.52^{bc}	1.23^{de}	$0.56b^{c}$	3.02^{abc}
9.	MRIZP-748	60.00^{bcd}	17.77^{bcd}	13.41^{bc}	2.63^{b}	0.600^{bc}	2.40^{cd}
10.	MRIZP-830	64.46^{bcd}	13.13^{d}	13.18^{bc}	1.80^{bcde}	0.633^{abc}	2.48^{cd}
	Average	57.64	16.43	13.30	1.67	0.629	2.85
11.	Control	95.53^{a}	31.62^{a}	22.49^{a}	3.97^{a}	1.067^{a}	0.10^{e}
	LSD (0.05)	3.574	6.864	5.411	0.800	0.388	0.898
	LSD (0.01)	4.876	9.364	7.382	1.092	0.529	1.225

* Values of column followed by the same letter(s) are not significantly different (P = 0.05) according to Duncan's multiple range test.

Our results on pathogenicity of *F. verticillioides* are in accordance with the results obtained by Desjardins et al. (1995) and Munkvold and Carlton (1997).

Vegetative compatibility

Mutants *nit1* and NitM, with prevalence of *nit1* (58.79%) over NitM (5.77%) (Table 5), were isolated from the observed isolates of *F. verticillioides*. According to the literature data (Klittich and Leslie, 1988), the frequency of mutants *nit1* is higher than the frequency of other types of *nit* mutants.

Tab. 5 — Frequency of *nit1* and NitM mutants in the studied *F. verticillioides* isolates

Isolate	*nit1* (%)	NitM (%)
MGA-7	92.86	4.29
MGD-4	70.00	15.00
MGE-5	55.00	5.00
MGG-13	40.00	5.00
MGI-1	65.00	5.00
MRIZP-201	45.00	6.67
MRIZP-237	62.50	1.25
MRIZP-570	50.00	7.14
MRIZP-748	67.50	5.00
MRIZP-830	40.00	3.33
Average	58.79	5.77

Ten vegetative compatible groups (VCGs) of *F. verticillioides* were established on the basis of the complementation test among the isolates in all possible combinations. These results point out to a high genetic diversity of the population of this fungus pathogenic to maize. Similar results were stated by Chulze et al. (2000).

CONCLUSION

All studied *F. verticillioides* isolates originated from wheat (five isolates) and maize grain (five isolates) expressed pathogenicity to maize seedlings. According to the percentage of nonemerged plants, it was established that six, three and one isolates expressed low, moderate and high pathogenicity. The survival rate (%) and vigour (growth and dry weight of roots and epicotyls) of plants that were developed from inoculated seeds, were significantly reduced (approximately two times) in comparison with the control. There was a tendency for isolates from different hosts to have similar values for pathogenicity. These results are of a practical importance from the aspects of maize and wheat crop rotation and for the success of breeding for resistance to *F. verticillioides*.

The analysis of the results on pathogenicity obtained on the basis of the scale for the % nonemerged plants, plant survival rate (%), vigour (growth and dry weight of roots and epicotyls) and disease severity, shows a concurrence in defining moderate and high pathogenicity of isolates, while there was a certain nonconformance among these results in relation to low pathogenicity defining. Nevertheless, a selection of parameters, such as the scale for % of nonemerged plants, is a simple and good choice for the characterisation of pathogenicity degree of all *F. verticillioides* isolates.

Two types of *nit* mutants, *nit1* and NitM, were obtained by the use of the method of vegetative compatibility. The frequency of *nit1* mutants was greater (58.79%) than the frequency of NitM mutants (5.77%). A total of 10 VCGs of *F. verticillioides* were determined in the complementation tests. This number of vegetative compatible groups indicates a high genetic diversity of the observed *F. verticillioides* population.

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ПАТОГЕНОСТ И ДИВЕРЗИТЕТ ВЕГЕТАТИВНЕ КОМПАТИБИЛНОСТИ *FUSARIUM VERTICILLIOIDES*

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Резиме

Патогеност 10 изолата *F. verticillioides*, пореклом из зрна пшенице (5 изолата) и кукуруза (5 изолата), проучавана је у условима стакленика. На основу различитих параметара оцене патогености (скала за % неизниклих биљака, % преживелих биљака, вигора биљака — пораст и сува тежина корена и епикотила, и интензитета болести) установљено је да су сви испитивани изолати *F. verticillioides* испољили различит степен патогености. Према % неизниклих биљака 6 изолата испољило је ниску, 3 изолата средњу и један изолат *F. verticillioides* високу патогеност. Сви испитивани изолати *F. verticillioides* су проузроковали смањено преживљавање и вигор биљака, са интензитетом болести од 2.0 до 3.54. Применом методе вегетативне компатибилности изоловане су две врсте *nit* мутаната, *nit1* и NitM. Учесталост мутаната *nit1* је била већа (58.79%) у односу на NitM мутанте (5.77%). У комплементарним тестовима установљено је 10 вегетативно компатибилних група (VCG) *F. verticillioides* што указује на висок генетички диверзитет популације овог патогена.