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THE DETERMINATION OF *EXSEROHILUM TURCICUM* VIRULENCE FACTORS IN SERBIA

Jelena LEVIĆ, Slavica STANKOVIĆ and Tijana PETROVIĆ

Maize Research Institute, „Zemun Polje“ - Belgrade, Serbia

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The determination of *Exserohilum turcicum* virulence factors and resistance responses of three sets of maize inbred lines (four differential, eight isogenic and 22 commercial inbreeds) to three isolates of this pathogen under greenhouse conditions were studied. The maize inbreeds were selected according to previous testing of resistance based on lesion types in 194 inbreeds under field conditions of plant inoculation with the *E. turcicum* race 0 (designated as the isolate MRI-Et). The standard procedure was applied to obtained isolates MRIZP-1747 and MRIZP-1416 from resistant and susceptible lesion types, respectively. These lesions were developed on the same leaf of a plant of the experimental hybrid no. 163/99 grown in a nursery at Zemun Polje during 1999. The third isolate (MRIZP-1435) was isolated from a leaf sample originating from the location of Srbobran in which the occurrence of northern corn leaf blight (NCLB), caused by *Exserohilum turcicum*, was intensive. Based upon virulence/avirulence of three isolates of *E. turcicum*

Corresponding author: Jelena Lević, Maize Research Institute, Zemun Polje, S. Bajića 1, 11185 Belgrade, Republic of Serbia, Tel.: ++ 381/11/3756704, Fax: ++ 381/11/3756707, e-mail: jlevic@mrizp.co.yu

on differential maize inbred lines, it was found out that the isolate MRIZP-1747 could be classified as race 0, whereas isolates MRIZP-1416 and MRIZP-1435 could be classified as race 1. These are the first results that confirm the presence of race 1 of *E. turcicum* in Serbia. Not including differential lines, 22 and six lines were resistant to race 0 and race 1, respectively, while eight and five lines were resistant and susceptible to both races, respectively. All isogenic lines not containing the *Ht* gene were susceptible to both races 0 and 1.

Key words: *Exserohilum turcicum*, race 0 and race 1, *Zea mays* L.

INTRODUCTION

The theoretical and practical research of maize resistance to races of *Exserohilum turcicum* (Pass.) Leonard & Suggs (syn. *Helminthosporium turcicum* Pass.) is in the control of this pathogen causing economically important maize leaf disease, Northern Leaf Blight (NLB). Eleven races of *E. turcicum* (R0, R1, R12, R123N, R12N, R1N, R2, R23, R23N, R2N and RN) have been identified worldwide (BERGQUIST and MASIAS, 1974; THAKUR *et al.*, 1989; WINDES and PEDERSON, 1991; LEONARD, 1993; GIANASI *et al.*, 1996; MWANGI, 1998). The classification proposed by LEONARD *et al.* (1989) and results obtained after mating of races under controlled conditions (FALLAH MOGHADDAM and PATHAKY, 1994) left room for the accommodation of other new races of *E. turcicum*. This statement was confirmed when OGLIARI *et al*. (2005) discovered two new races (R123x and R23rx).

The first report on *E. turcicum* reported in Serbia in 1925 was associated with Northern leaf blight epidemics in the maize growing region (JOSIFOVIĆ, 1926). This pathogen used to cause enormous damage from the mid 1950s to the mid 1960s, in the lowland regions of Serbia. It was coincided with humid conditions and the wide growing of susceptible maize hybrids, such as introduced hybrids Wisconsin 692, Ohio C-92 and Kansas 1859 (PENČIĆ and MARIĆ, 1962). Since the mid 1960s, the disease has been effectively controlled by the development of the hybrids resistant to *E. turcicum*. Until the late 1970s the majority of hybrids used had a polygenic type of resistance. Afterwards, the dominant *Ht* genes, mostly Ht_1, are incorporated into new hybrids.

During the last two decades, the identification of *E. turcicum* virulence factors in Serbia was studied on the basis of the response of differential maize inbred lines carrying *Ht* genes. Results showed that only the race 0 existed. However, in 1999 at Zemun Polje and Srbobran, a susceptible response to *E. turcicum* was observed in a genotype carrying the Ht_1 gene and a genotype with unknown background with a high incidence of NLB, respectively. At Srbobran, the severity of NLB was 3 on the 0-5 rating scale, where 0 means no symptoms while 5 means that all leaves are infected, lesions merged. After 20 years of the maize production monitoring, this was the first case of a more intensive NLB epiphytotic the severe occurrence of NLB in 1999 could be explained by lower temperatures

and higher precipitation than the long-term mean in the growing season (July-August).

Detecting only a few lesions in some locations has been quite common, but detecting them each year would have been very unusual. The cultivation of resistant genotypes and weather conditions (warm and dry summers) are reasons NCLB has not been a significant foliar disease of maize in Serbia until recently.

The objective of this study was to determine *E. turcicum* virulence factors and resistance responses of 34 maize inbred lines to three isolates of this pathogen. These isolates were studied only under controlled greenhouse conditions with the aim to avoid the increase of the inoculum in the environment.

MATERIAL AND METHODS

Plant materials. Thirty-four maize inbred lines were selected from194 inbreeds inoculated with *E. turcicum* race 0 (designated as isolate MRI-Et) in preliminary tests carried out in the nursery at the Maize Research Institute, Zemun Polje. Inbred lines were selected according to the resistance or susceptibility of lesions. Selected inbred lines were divided into three sets: four differential lines, eight isogenic lines and 22 commercial lines (Table 1). A set of differential maize inbred lines such as A632Ht_1, A619Ht_2, H95Ht_3 and A632*HtN* was developed by A.L. HOOKER from the University of Illinois.

Plants were successively inoculated two times in 7 to10day intervals. The first inoculation was performed in the 5 to7 leaf stage or when the plant height amounted to 50 to60 cm, and the second prior to tassel emergence. Plants were inoculated by placing ground leaf tissue previously inoculated by MRI-Et isolate (race 0) into the leaf whorls.

Source of isolates. Three selected *E. turcicum* isolates were re-inoculated onto 34 maize inbred lines. Resistant and susceptible types of lesions caused by *E. turcicum* on the same leaf of the experimental hybrid no. 163 (Fig. 1) were established under field nursery conditions of the Maize Research Institute at Zemun Polje (MRIZP), Serbia, in 1999. This leaf was collected and different lesions were separately cut, sterilised in a 0.5% sodium hypochlorite solution (1:3, v/v) for 1 minute, washed three times with sterile water and placed between two sterile towel papers to remove excess water. Small fragments (2–3 mm) of sterilised leaf lesions were cut from the edge of chlorotic/necrotic and healthy tissues, placed on the PDA medium and incubated at 25ºC in the dark. Monoconidial cultures were obtained from PDA isolates by transferring a single conidium to carnation leaf agar (FISHER *et al*., 1982). Cultures obtained from resistant and susceptible lesion types were designated as isolates MRIZP-1747 and MRIZP-1416, respectively. By the application of this procedure, the culture designated as MRIZP-1435 was isolated from the leaf with a susceptible type of lesions that was sampled in the location of Srbobran in 1999.

These cultures were used as inoculum sources for determination of *E. turcicum* virulence (races) and responses of maize inbred lines. The spore concentrations were approximately 10^4 spores per ml determined by the use of a hemocytometer. Two replicate experiments were performed in the MRIZP greenhouse.

Determination of virulence factors. To confirm the race identity, avirulence/virulence of MRIZP-1747, MRIZP-1416 and MRIZP-1435 on four inbred lines carrying different dominant *Ht* genes was assessed under greenhouse conditions.

Plastic containers (27 x 15 cm) were filled with the Klassman mixture, sand and clay (1:1:1, v/v/v) and 10 seeds per entry were sown. The treatment was replicated three times. Plants were inoculated with the fungal spore suspension in the 4-leaf stage for the first time, and then repeated after a one week interval using a small hand held sprayer.

The lesion type was assessed 18 and 27 days after inoculation with isolates MRIZP-1747, MRIZP-1416 and MRIZP-1435 using the method of OGLIARI *et al*. (2005). According to this method resistant plants reactions were R_1 (no lesion), R_2 (chlorotic points or small round chlorotic-necrotic lesion) and R_3 (narrow chlorotic-necrotic lesions at the initial developmental phase). Susceptible plants produced straw coloured olive green necrotic lesions and their reactions were S_5 (necrotic lesions with no chlorotic halo circumscribed with a dark border at the edge) or S_6 (necrotic lesions with no circumscription with dried and shrunken edge of the leaves). Results of this assessment were used for characterisation of the virulence factors of *E. turcicum* isolates using the classification system of LEONARD e*t al.* (1989).

Response of maize inbreeds. The studies encompassed the second and the third set of lines, which previously showed a resistant or chlorotic type of lesions after artificial inoculations of plants with MRI-Et isolate of *E. turcicum* (race 0) in the experimental field of the MRIZP. The substrate preparation, sowing, inoculum production, inoculation and lesion type examination were carried out as described above.

RESULTS

The morphological observation showed that two isolates of *E. turcicum* had similar conidial traits (Fig. 1b, 1c), but the isolate MRIZP-1747 sporulated better on PDA medium than the isolate MRIZP-1416.

Individual analysis of each *E. turcicum* isolate showed that the isolate MRIZP-1747 produced chlorotic lesions of the resistant type in the lines A632Ht_1, A619Ht_2, H95Ht_3 and A632*HtN* (Table 1). Such behaviour suggested that the isolate MRIZP-1747 was avirulent on four inbred lines carrying different dominant *Ht* genes. Similar results were obtained under field conditions when these inbreeds had been inoculated with the *E. turcicum* isolate designed as MRI-Et.

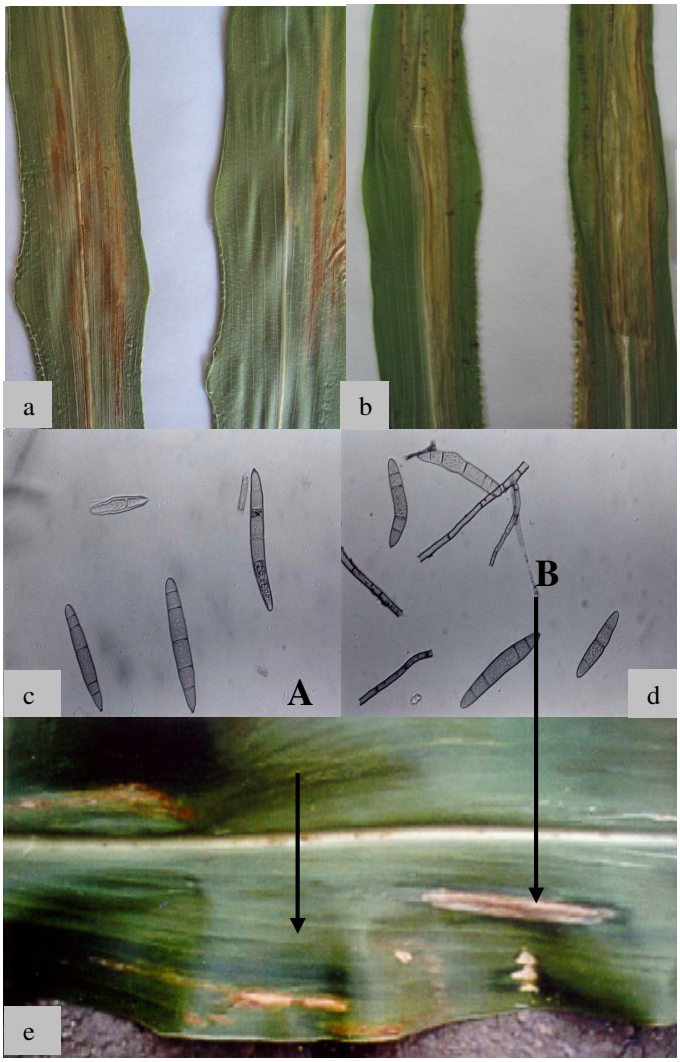

Figure 1. *Exserohilum turcicum*: response of inbred line Va36 to the isolate MRIZP-1747 (a) and the isolate MRIZP-1416 (b). conidia of the isolate MRIZP-1747 (c) and the isolate MRIZP-1416 (d); lesion types caused by the isolate MRIZP-1747 (A) and the isolate MRIZP-1416 (B) on the same leaf of one maize genotype in the field (e);

These results indicated that the isolate MRIZP-1747 had a virulence formula *Ht1Ht2Ht3HtN/*, known as the formula for race 0 of *E. turcicum*.

The analysis of the isolate MRIZP-1416 showed that it produced susceptible lesions in maize line A632Ht_1 and resistant lesions in lines A619Ht_2, H95Ht_3 and A632*HtN*. These results suggest the virulence formula of this isolate is *Ht2Ht3HtN/Ht1* (ineffective/effective pathogen genes, respectively). These isolates can also be classified as the race 1 based on the nomenclature proposed by LEONARD et al. (1989).

Table 1. Resistance and susceptibility reaction[a] of maize lines inoculated with Exserohilum turcicum isolates MRI-Et, MRIZP-1747, MRIZP-1416 and MRIZP-1435

Line designation	Related/Derivation	Field conditions[b]	Greenhouse conditions[c]		
		MRI-Et	MRIZP -1747[d]	MRIZP-1416	MRIZP-1435
Differential lines					
A632Ht_1	A 632 (BSS)	R	R	S	S
A619Ht_2	A 619 (LSC)	R	R	R	R
H95Ht_3	H 95 (Lancaster)	R	R	R	R
A632*HtN*	A 632 (BSSS)	R	R	R	R
Isogenic lines					
A632	Mt42 x B 14 (BSS)	S	S	S	S
A632*Htwx*	A 632 (BSS)	R	R	S	S
A619	A 171 x Oh 43 (Lancaster)	S	S	S	S
B14	(BSS)	S	S	S	S
B14Ht_2	B 14 (BSS)	R	R	R	R
B73	(BSS)	S	S	S	S
B73Ht_1	(BSS)	R	R	S	S
H95	Oh43 x C 190A (Lancaster)	S	S	S	S
Commercial lines					
AS58	B 73 x N152 (BSS)	R	R	S	S
D103	CM 105 & B84 (BSS)	R	R	R	R
D104	CM 105 & B84 (BSS)	R	R	S	S
E1041	(BSS)	R	R	S	S
H93 Ht_1	B 73 x GE 440 (BSS)	R	R	S	S
L12b	A 654 (Unrelated)	R	R	R	R
L1580	(BSS)	R	R	S	S
L1584	(BSS)	R	R	R	R
L518A	Yu O.P.V.[e]	R	R	R	R
L620*su*	(BSS)	R	R	R	R
L70/9	Mo17 (Lancaster)	R	R	S	S
L732	Yu O.P.V.[e]	R	R	S	S
L793	(BSS)	R	R	S	S

Continuated table 1

V312	Yu O.P.V.[e]	R	R	R	R
Va35	(C 103 x T8)T8 (Lancaster)	R	R	S	S
Va36	(C 103 x T8) (Lancaster)	R	R	S	S
W153R	Rec. I. 153R (Unrelated)	R	R	S	S
W37A	Win. 25# x A 374	R	R	S	S
Wf 9	Reid Yellow Dent (O.P.)	R	R	S	S
ZPL720	Yu O.P.V.[e]	R	R	S	S
ZPL198	Ar O.P.V.[f]	R	R	R	R
ZPP4-1	Yu O.P.V.[e]	R	R	S	S
E. turcicum virulence		0	0	1	1

[a] R – resistant plants with R_1, R_2 or R_3 reactions, S – susceptible plants with S_5 and S_6 reactions;

[b] Plants inoculated with ground leaf infected with isolate MRI-Et;

[c] Plants inoculated with spores suspension of fungal pure culture;

[d] Number of pathogen culture collection in Maize Research Institute „Zemun Polje“, Belgrade-Zemun, Republic of Serbia;

[e]Yugoslav open-pollinated variety;

[f]Argentinean open pollinated variety.

BSSS Iowa Stiff-Stalk Synthetic

Five isolates of *E. turcicum*, including field and greenhouse studies, were effective against maize genotypes lacking all resistance genes, such as isogenic inbreeds A632, A619, B14, B73 and H95. All of these isolates were ineffective against line B14Ht_2 and isolates MRIZP-1416 and MRIZP-1435 were also ineffective against isogenic line A632*Htwx*.

All lines within the third set of selected lines were resistant to the isolate MRIZP-1747, as it was a case in the previous test performed in the field with the isolate MRI-Et, which had been classified as the race 0. Out of all these lines only line H93Ht_1 had a known background, as the gene resistant to the *E. turcicum* race 0 had been introduced by the cross of the inbred B73 to GE440. Namley, HOOKER (1961) was the first author who described a chlorotic or resistant type of resistance in the inbred GE440 and popping maize Ladyfinger. This resistance was controlled by only one dominant gene, designated with Ht_1 or just *Ht* (HOOKER, 1963), which is positioned on the chromosome 2L bin 2.0 (BENITOLILA *et al*., 1991).

Seven and 15 out of these lines expressed resistance and susceptibility, respectively, to both, isolates MRIZP-1747 and MRIZP-1435. Lines derived by crosses to local (Yugoslav) varieties or bred from them are especially interesting, as they carry resistance to studied isolates, which were, according to our research, classified as the *E. turcicum* races 0 and 1.

DISCUSSION

The *E. turcicum* races are defined on the basis of their phenotypic reactions after inoculation of a set of differential maize lines. The race 0 (MRI-Et and MRIZP-1747) is ineffective (avirulent) against all *Ht* genes (Ht_1, Ht_2, Ht_3 and *HtN*), whereas the race 1 (MRIZP-1416 and MRIZP-1435) is only effective (virulent) against Ht_1. The *E. turcicum* race classification, based on their avirulence and virulence on the four different dominant *Ht* genes, was proposed by LEONARD *et al.* (1989). According to the earlier classification, races 0, 1, 23 and 23N corresponded to races designated as 1, 2, 3 and 4. The identification of these races is rapid under controlled conditions due to inoculated isogenic lines responses (LEATH *et al.*, 1990).

The race 0 is predominant worldwide and the economically most important race of *E. turcicum* race, followed by the race 1. The occurrence of races 23, 2N or 23N is rare (FALLAH MOGHADDAM and PATAKY, 1994), although the race R23N has displayed a tendency to spread in the United States (FERGUSON and CARSON, 1998). WELZ *at al*. (1993) identified the presence of races 0 and 1 in China, races 23, 23N and R2N in Mexico, races 0, 23 and R23N in Zambia, and races 0, 2, N and 23N in Uganda. According to BIGIRWA *et al.* (1993) only the race R0 was found to be prevalent in Uganda GIANASI *et al.* (1996) discovered races N, 1N, 12N, and 123N on the basis of the resistance of maize inbred lines B73*Ht1*, B73*Ht2*, A619*Ht3*, B73*HtN* and M66 to 25 Brazilian isolates of *E. turcicum*.

Detailed studies of a physiological specialisation of *E. turcicum* in Kenya indicated the presence of races 0, 1, 12, 2, 23 and nine isolates, that did not fitting any known classification race, and were designated named as k1, k2 and k3 (MWANGI, 1998). Those classified as k1 closely resembled the race 4 (R123N), but were virulent on the Ht_1 gene. The same author concluded that the classification system established by LEONARD et al. (1989) was not inclusive and did not satisfy the naming of all the isolates.

In Brazil, OGLIARI *et al*. (2005) two races based on their virulence/avirulence in differential lines were identified. The race designated as 123x was effective on lines with Ht_1, Ht_2 and Ht_3 genes. On the other hand, the second race designated as 23rx, was virulent on lines with Ht_1 and *HtN* genes, while was aviruelent on lines with Ht_2 and Ht_3 genes.

The race divergence of *E. turcicum* in Europe was not as pronounced as above described. It was also confirmed by studying European populations of *Setosphaeria turcica* Leonard & Suggs (anamorph *E. turcicum*) on the molecular level (BORCHARDT *et al*., 1998).

In Europe, a new race of *E. turcicum* was established on the basis of a susceptible response of genotypes with the Ht_1 gene in Croatia (PALAVERŠIĆ and LENDLER, 1996) and Slovenia (ROZMAN *et al.*, 2003). Results obtained on the basis of responses of differential maize inbred lines and presented in this study confirm the existence of races 0 and 1 in Serbia.

Results suggest that inbred maize lines resistant to the race 0 and susceptible to the race 1 (Fig. 1d, e) carry the Ht_1 gene, while inbreds resistant to both races probably carry genes different from the Ht_1 gene.

These findings suggest that more studies on the racial specialisation in Serbia are needed and that in regard to the NLB management, it is necessary to incorporate other *Ht* genes besides Ht_1, which has been intensively used since the 1980s. Resistant genotypes described in this study could be used as sources of resistance in the breeding programme, especially genotypes genetically different from Ht_1, but resistant to both *E. turcicum* races, 0 and 1. Open-pollinated varieties (Yugoslav and Argentinean) are particularly interesting as a source of resistance to these races. As early as the 1980s, LEVIĆ *at al*. (1983) and PENČIĆ and LEVIĆ (1981), working with local varieties, observed that the Yugoslav varieties were a good source of monogenic resistance to *E. turcicum*, especially from the group of dent types of the USA Corn Belt dents that were most often used for deriving maize inbreds and ZP hybrids.

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UTVRĐIVANJE FAKTORA VIRULENTNOSTI *EXSEROHILUM TURCICUM* U SRBIJI

Jelena LEVIĆ, Slavica STANKOVIĆ i Tijana PETROVIĆ

Institut za kukuruz, Zemun Polje, Beograd-Zemun, Republika Serbia

I z v o d

U ovom radu proučavani su faktori virulentnosti *Exserohilum turcicum* i tipovi otpornosti tri seta samooplodnih linija kukuruza (četiri diferencijalne, osam izogene i 22 komercijalne linije) prema tri izolata ovog patogena u uslovima staklare. Linije su izabrane na osnovu prethodnog testiranja tipa otpornosti pega kod 194 samooplodnih linija u poljskim uslovima inokulacije biljaka rasom 0 vrste *E. turcicum* (označena kao izolat MRI-Et). Standardnim postupkom je iz otpornog tipa pege izolovana kultura izolata MRIZP-1747, a iz osetljivog tipa pege izolat MRIZP-1416. Ove pege su bile obrazovane na istom listu jedne biljke eksperimentalnog hibrida br. 163/99 u rasadniku kukuruza u Zemun Polju u 1999. godini. Izolat MRIZP-1435 je iste godine izolovan iz uzorka lista kukuruza poreklom iz lokaliteta Srbobran u kojem je bila neoubičajeno intenzivna pojava sive pegavosti lista koju prouzrokuje *E. turcicum*. Na osnovu virulentnosti i avirulentnosti tri izolata *E. turcicum* na diferencijalnim linijama kukuruza utvrđeno je da se izolat MRIZP-1747 može klasifikovati kao rasa 0, dok se izolati MRIZP-1416 i MRIZP-1435 mogu klasifikovati kao rasa 1. Ovo su prvi rezultati koji potvrđuju da u Srbiji osim rase 0 postoji i rasa 1 *E. turcicum*. Ne uključujući diferencijalne linije, otpornost prema rasi 0 ispoljile su 22 linije i šest linija prema rasi 1. Istovremeno, osam linija je bilo otporno prema rasi 0 i pet linija prema rasi 1. Sve izogene linije koje nisu sadržavale *Ht* gen bile su osetljive prema ovim rasama.

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