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Scientific paper

Pathogenicity of *P. terrestris* on Maize Seedlings

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SUMMARY

Pathogenicity of *P. terrestris* was determined by the Knop's medium slants method in test tubes. Isolates originated from the roots of maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), Johnson grass (*Sorghum halepense* Pers.), sorghum (*Sorghum bicolour* (L.) Moench.), garlic (*Allium sativum* L.), onion (*Allium cepa* L.), barnyard millet (*Echinochloa crus-galli* (L.) P.Beauv.) and green foxtail (*Setaria viridis* (L.) P.B.). A fragment of a fungal colony, cultivated on PDA, was placed on the bottom of Knop's medium slant in each test tube and then sterilised a maize seed was placed 2 cm away from the inoculum. After 21-day inoculation of seeds, the intensity of the development of symptoms on maize seedlings was estimated. The reddish or dark pigment on the root, mesocotyl and/or coleoptyl of seedlings was an indicator for the infection by the fungus under *in vitro* conditions. Based on the pathogenicity test, the isolates were classified into the following three groups: slightly (3 isolates), moderately (6 isolates) and very pathogenic (6 isolates) to maize seedlings. The obtained results show that *P. terrestris*, originating from different hosts, can be a maize pathogen. These results can explain the high frequency and high incidence of this fungus on maize roots in Serbia.

Keywords: *P. terrestris*; Maize seedlings; Pathogenicity

INTRODUCTION

Pyrenochaeta terrestris Gorenz, Walker & Larson (syn. *Phoma terrestris* Hansen) is widespread in the world and causes red root rot of many plants, including cultivated and weed species (Farr et al., 1989). This fungus is most often and in detail described in literature as an onion pathogen. It survives to a depth of 18 inches in soil and is associated with soil particles 0.5 to 1.0 mm in diameter (Siemer and Vaughan, 1971) or with plant debris particles (Sneh et al., 1974). This fungal pathogen is spread through infected soil, plant debris, water and by mechanical cultivation. Dehydrated cultures of *P. terrestris* are vital even after 13 months (Gorenz et al., 1948).

In Serbia, this fungus was initially identified on onion roots (Aleksić et al., 1989), and then in 1996 it was identified on roots of maize, wheat, barley, oat, sorghum and Johnson grass, barnyard millet, green foxtail, garlic and onion (Petrović and Lević, 1999). *P. terrestris* developed very early in the maize root, hence its incidence varied from 29.5% to 58.1% in the stage of

intensive vegetative development under the agroecological conditions in Serbia (Lević et al., 2011). A higher incidence of this fungus was found in the root of early maturity maize genotypes than in late maturity genotypes. Although, *P. terrestris* is widespread and develops on a greater number of hosts, it has been rarely isolated and little work has been done to study it in Serbia. One of the reasons for this is a weak sporulation of the fungus on standard medium, such as potato dextrose agar. Moreover, the fungus is not easily recognized and the identification of cultures is time- and labour-consuming.

Even though *P. terrestris* is described in literature as a pathogen of maize root in the field, there is little data on the pathogenicity of this fungus on maize plants. Because of this, and the fact that the fungus occurs on roots of many plant species that are associated with maize, the aim of the present study was to observe pathogenicity of isolates originating from roots of different plants species to maize seedlings.

MATERIAL AND METHODS

Identification and maintenance of the fungus

The tested isolates were obtained from roots of eight different plant hosts: maize (7 isolates), barley (1 isolate), Johnson grass (1 isolate), sorghum (1 isolate), garlic (2 isolates), onion (1 isolate), barnyard millet (1 isolate) and green foxtail (1 isolate). The identification of *P. terrestris* was confirmed by transferring aseptically a hyphal tip of the fungus radiating from the root section on the surface of three media: potato dextrose agar (PDA), medium with a sterile carnation leaf fragments (CLA) and a synthetic nutrient-poor agar (SNA). The mycelium was transferred from each Petri dish from three sections with a red purple pigment for which it was assumed that it was a result of *P. terrestris* (Figure 1). Two media, CLA and PDA, were prepared after Burgess et al. (1994), while the medium SNA was prepared following the method developed by Nirenberg (1976). The fungus was grown on PDA at 25°C in the dark and on CLA and SNA in the alternating 12 h combined light (fluorescent and near ultra violet (NUV) light) and 12 h dark conditions. Based on the previously described properties of the fungus on PDA and CLA media (Lević et al., 2011) and the characteristic red to red purple colour (beet-red) of filter paper on the SNA medium, the isolates were identified as *P. terrestris*.

Pathogenicity test

Determination of *P. terrestris* pathogenicity was performed in test tube according to a method developed at the Maize Research Institute, Zemun Polje, Belgrade, Serbia.

For inoculation, *P. terrestris* isolates were cultured in PDA on 9-cm diameter Petri dishes for 7 days at 25°C. The slant Knop's medium, prepared according to Tuite (1969) was placed in 160 x 20 mm test tubes. This medium contains 0.8 g $Ca(NO_3)_2 \bullet 4H_2O$, 0.2 KNO_3, 0.2 g KH_2PO_4, 0.2 $MgSO_4 \bullet 7H_2O$, in trace $FeSO_4$ and 20 g agar, 500 ml water. A 4-mm PDA fungal plug was placed 2 cm away from the bottom of each test tube on the surface of Knop's medium slants.

Maize seeds were surface-sterilised in 1% sodium hypochlorite solution for 5 min, washed three times with distilled water, dried on soft paper and then sterilised seeds were placed 15-20 mm above the inoculum site. The seeds were placed with the embryo facing the medium and carefully pressed into the bead surface. The test tubes were closed with cotton wool and then placed vertically in a slotted metal basket and held for three weeks under room conditions. A three-replicate test was performed with 10 seeds per each replication.

Light or dark pink, dark purple to red brown tissues were visually estimated on the primary roots, seminal roots, mesocotyls and coleoptiles of 21-day-old maize seedlings. Disease ratings were recorded using a 0-5 scale, where 0 = healthy seedlings and 5 = severely discoloured roots, mesocotyls and coleoptiles or ungerminated seeds. Pathogenicity of all maize isolates was measured as a disease severity index (DSI) according to the following equation (Korolev et al., 2008): $DSI = [\Sigma(R \times N)] \times 100/H \times T$, where R = disease rating, N = number of plants with this rating, H = highest rating category, i.e. 5, T = total number of plants counted. Based on DSI data, the isolates of *P. terrestris* were classified as low (0 to ≤ 30 DSI), moderately (≥31 to ≤ 60 DSI) and highly (over 60% DSI) pathogenic to maize seedlings.

RESULTS AND DISCUSSION

In the pathogenicity test *in vitro*, light pink to deep pink discoloration of root tissue (primary and seminal roots) and the coleoptile of 21-day-old maize seedlings was the most frequent symptom caused by *P. terrestris* isolates (Figure 1). Also, red brown to brown spots or lesions were formed, more on the root of seedlings than on the coleoptile. Furthermore, a slight reduction in root growth was recorded in tests of some isolates, particularly those originating from the roots of maize (up to 15%).

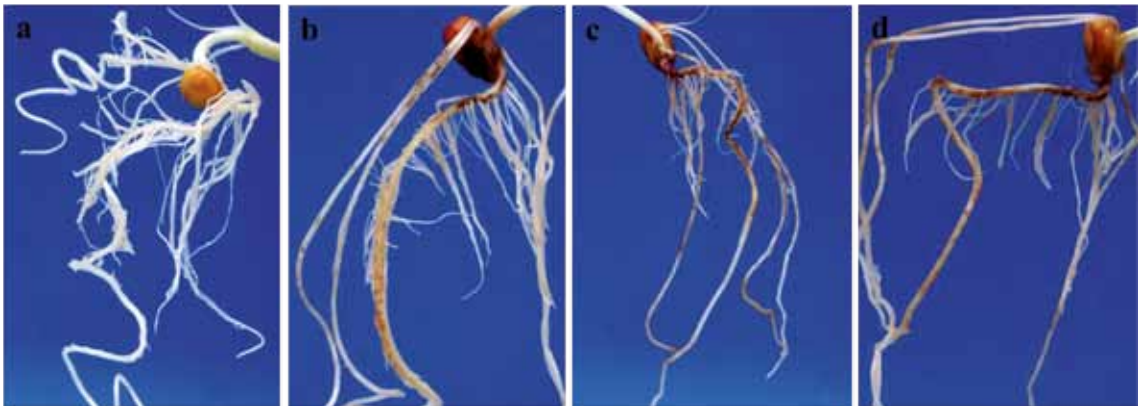

Figure 1. Symptoms of red root rot (*Pyrenochaeta terrestris*) on maize seedlings (b-d) and control (a). Symptoms caused by slightly (b), moderately (c) and very pathogenic isolates of *P. terrestris*.

Three out of 15 *P. terrestris* isolates were slightly pathogenic (DSI from 24.0 to 30.0%), six isolates were moderately pathogenic (DSI from 37.5 to 59.5%) and six isolates were very pathogenic (DSI from 71.0 to 90.9%) (Table 1).

The highest DSI values were observed in the case of inoculation with the K16/2-1 isolate originating from maize root (90.9%) and with the KSh-45 isolate originating from sorghum root (86.0%).

In literature, pink colour is usually listed as a symptom developed by *P. terrestris* on the roots of seedlings. However, our results are in accordance with Carvajal (1945) who described the tissue affected by *P. terrestris* turning red-brown. Application of the soil inoculation method resulted in necrotic root tissue in approximately 25% of inoculated plants (Koenning et al., 2007). According to these authors, approximately 90% of inoculated plants had discoloured crowns that resembled symptoms on field infected plants. Stalk inoculations resulted in necrosis extending 2 to 5 cm from the point of injection, and in shoot death of 40% of inoculated plants that developed adventitious shoots. Sumner et al. (1990) found that *P. terrestris* caused a slight to moderate root disease, but had little or no effect on seedling growth under greenhouse conditions. Our results show that the fungus slightly reduced the root growth of maize seedlings. According to Sumner et al. (1985), *P. terrestris* caused the most severe root rot in the greenhouse pathogenicity test, as we found in the test tube.

Table 1. Mean rating and disease severity index (DSI) in 21-day-old maize seedlings caused by *P. terrestris*

No.	Isolate	Source - plant root	Mean rating[a]	DSI[b] (%)	Level of pathogenicity
1.	Ac-42	*Allium cepa* L.	1.5	30.0	Low
2.	As 29	*Allium sativum* L.	1.2	24.0	Low
3.	As-20	*Allium sativum* L.	3.8	75.6	High
4.	KPcg-2	*Echinochloa crus-galli* (L.) P. Beauv.	2.1	42.0	Moderate
5.	Hv-26	*Hordeum vulgare* L.	2.3	46.3	Moderate
6.	KSv-3	*Setaria viridis* (L.)P.B.	3.6	71.0	High
7.	Ksir-86	*Sorghum bicolor* (L.) Moench.	3.8	75.6	High
8.	KSh-45	*Sorghum halepense* Pers.	4.3	86.0	High
9.	Pt-1	*Zea mays* L.	1.3	26.0	Low
10.	677KIII/5-6	*Zea mays* L.	1.6	37.5	Moderate
11.	704KII/5-6	*Zea mays* L.	2.0	40.7	Moderate
12.	539KII/1-12	*Zea mays* L.	2.3	45.6	Moderate
13.	KKR-43	*Zea mays* L.	3.0	59.5	Moderate
14.	K1/4	*Zea mays* L.	3.9	78.0	High
15.	K16/2-1	*Zea mays* L.	4.5	90.9	High
	Mean		2.7	55.2	

[a]Mean rating of disease intensity occurrence on a 0-5 scale
[b]DSI = Sum of (Disease rating × number of plants with this rating) x 100/ highest rating category × total number of plants countered.

P. terrestris isolates originating from roots of red onion, garlic, alfalfa, soil under sedge, rice and cotton, express pathogenicity towards maize, which shows that there is no physiological specialisation of the fungus. In addition, Carvajal (1945) has established that there were no differences in cultural and morphological properties of the isolates originating from sugarcane, red onion, garlic, alfalfa, maize, rice and cotton. This author used a method by which a small piece of agar, bearing the actively growing fungus, was placed at the tip of a 15-25-mm long root and incubated in the moist chamber at room temperature in the dark. Our results show that the isolates originating from the roots of different hosts were pathogenic to maize, but their aggressiveness differed.

In conclusion, the test tube method enabled us to easily determine the pathogenicity of *P. terrestris* isolates on the roots of maize seedlings. In general, the fungus isolates originating from the roots of eight different plant species were moderately pathogenic. The origin of isolates had no effect on their pathogenicity or virulence. These results may explain the wide spread of the fungus on maize root in Serbia.

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Patogenost *P. terrestris* na klijancima kukuruza

REZIME

Za utvrđivanje patogenosti izolata *P. terrestris* korišćena je metoda sa zakošenom Knopovom podlogom u test-epruveti. Poreklo izolata je sa korena kukuruza (*Zea mays* L.), ječma (*Hordeum vulgare* L.), divljeg sirka (*Sorghum halepense* Pers.), gajenog sirka (*Sorghum bicolour* (L.) Moench.), belog luka (*Allium sativum* L.), crnog luka (*Allium cepa* L.), korovskog prosa (*Echinochloa crus-galli* L.) i zelenog muhara (*Setaria viridis* (L.) P.B.). Fragment kolonije gljive, gajene na PDA, je stavljen na donji deo zakošene Knopove podloge u epruveti i 2 cm iznad toga sterilisano seme kukuruza. Nakon 21 dana od inokulacije semena ocenjen je intenzitet razvoja simptoma na klijanacima kukuruza. Crvenkast ili mrki pigment na korenu, mezokotilu i/ili koleoptilu klijanaca je bio indikator za infekciju gljivom u *in vitro* uslovima. Na osnovu testa patogenosti izolati gljive su grupisani u sledeće tri kategorije: slabo (3 izolata), srednje (6 izolata) i jako patogeni (6 izolata) za klijance kukuruza. Dobijeni rezultati ukazuju da je *P. terrestris*, poreklom sa različitih domaćina, patogen za kukuruz. Ovi rezultati mogu objasniti učestalost i intenzitet pojave ove gljive na korenu kukuruza u Srbiji.

Ključne reči: *P. terrestris*; klijanci kukuruza; patogenost