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Investigation of the mycelial compatibility of *Macrophomina phaseolina*

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Abstract

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Macrophomina phaseolina (Tassi) Goid. is found on all five crop-growing continents. In hot and dry seasons, this fungus is capable of causing considerable damage. In this study, mycelial compatibility of *M. phaseolina* isolates was investigated. In total the 30 samples collected were tested to examine their compatibility. The sunflower samples examined were collected in 2019 and 2020 in different regions of Hungary (29 isolates) and Slovakia (1 isolate). A total of 465 pairing tests were made with 30 isolates. The results of our examination showed incompatibility in 12 pairings. In our studies, we also measured the size of the microsclerotia of the isolates in order to determine which group they belong to. The diameter of the microsclerotia ranged from 74 to 182 µm. Based on this, microsclerotia belong to group 'C', as well as the data of previous studies in Hungary.

Keywords

genetic variability, host plant, invasive pathogen, *M. phaseolina*, sunflower, vegetative compatibility

Introduction

Macrophomina phaseolina (Tassi) Goid. (Botryosphaeriaceae) [synanamorph: *Rhizoctonia bataticola* (Taubenh.) E. J. Butler, nowadays used as one of its synonyms] is an important plant pathogenic fungus (Ambrósio et al., 2015). The pathogen has two anamorph stages. *Macrophomina phaseolina* is the name of the pycnidia form. Pycnidia generally develop the host tissues. The microsclerotial stage is named *Rhizoctonia bataticola,* which produces microsclerotia both on stems and within the stems.

M. phaseolina is considered to be an invasive fungus, with more and more hosts and can cause huge loss of yield to many crops worldwide (Dhingra and Sinclair, 1978). Under dry and risky conditions this pathogen can cause up to 100% crop loss (Damtea and Ojiewo, 2016). *M. phaseolina* has been reported in Italy (Zazzerini, 1980), Spain (Jimenéz-Diaz et al., 1983), Portugal (de Barros, 1985), Romania (Ionită et al., 1996), Serbia (Aćimović, 1998), Bulgaria (Alexandrov, 1999), Russia (Yakutkin, 2001), USA (Gulya et al., 2002), Czech Republic (Kudlíková et al., 2002), Slovakia (Bokor, 2007) and Turkey (Mahmoud and Budak, 2011).

In Slovakia the fungus caused a high rate of sunflower infection in 2005 and 2006 (Bokor, 2007). Jimenéz-Diaz et al. (1983) estimated that the charcoal rot and losses reached 20–36% in sunflowers. Veverka et al. (2008) reported that one of the critical questions for further research is to know how the pathogen survives both in the soil and/or on other hosts. The fungus overwinters by forming microsclerotia and tolerates low temperatures and can infect host plants easily in the following years (Awan et al., 2018). In Hungary, the pathogen was observed first on sunflowers in 1970 (Békési, 1970). Békési (2002) reported an infection rate of 90% in sunflowers, which resulted in a loss of yield of about 30–35%.

This invasive fungus has a wide host range including field crops, vegetables, trees, other herbaceous plants,

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weeds, and dicot or monocot plant species worldwide. *M. phaseolina* has been reported in many important plants such as *Ipomoea batatas* (Taubenhaus, 1913), *Solanum tuberosum* (Ashby, 1927), *Lycopersicon esculentum, Phaseolus vulgaris* (Small, 1928), *Helianthus annuus* (Mackie, 1932), *Zea mays* (Maneval, 1937), *Cucumis melo* (Wiant, 1937), *Medicago sativa* (Hoffmaster et al., 1943), *Glycine max* (Preston, 1945), *Nicotiana tabacum* (Reichert and Hellinger, 1947).

It was also found on weeds, for example *Ambrosia artemissifolia* (Boewe, 1963) and *Xanthium species* (Nikandrow et al., 1990), and also some ornamental plants *Crinum asiaticum* and *Hymenocallis littoralis* (Huda-Shakirah et al., 2019).

In Hungary, *M. phaseolina* was reported on *Zea mays* (Vörös and Manninger, 1973), *Glycine max* (Érsek, 1979), *Solanum tuberosum, Helianthus tuberosus, Phaseolus vulgaris, Vicia faba, Allium sativum* (Simay, 1987; Simay, 1990), *Beta vulgaris* (Koppányi et al., 1993), *Cannabis sativa, Valeriana officinalis* (Simay and Kadlicskó, 1993), *Capsicum annuum* (Fischl et al., 1995), *Citrullus lanatus* (Békési et al., 1995), *Prunus armeniaca* (Vajna and Rozsnyai, 1995) and *Picea pungens* (Fischl et al., 2008).

The pathogen is able to spread by seeds (Simay, 1990), or it can infect the host plants from the soil and stubble (Chaudhary et al., 2017). The pathogen causes charcoal rot, root rot and seedling blight (Small, 1928). Infected plants lose their vitality and wither and early death is typical (Csöndes, 2009). When the air temperature is high around the blooming period, symptoms are dramatic and progressive on many crops (Blanco-Lopéz and Jiménez-Diáz, 1983). The most dangerous weather conditions are drought and high temperature, and these conditions generate water stress for the plants (Mayer-Perez et al., 2002). A soil temperature of 28–35 °C is the most suitable for *M. phaseolina* infection (Dhingra and Sinlair, 1978). After blooming, the lower part of the stem and the top of the taproot show grey and black discoloration. Plant tissues are shredded (Csöndes, 2009), the stem epidermis regularly flakes away and inside the stem hundreds of microsclerotia are visible (Kolte, 1985). After infection on sunflowers the fungus can cause a decrease of 1,000 in seed weight and protein content. (Keerio et al., 2014). In Bulgaria, the charcoal rot caused 16 to 42.8% damage in the 1990–1996 period (Alexandrov, 1999). Control of the pathogen is complicated because soil tillage practices and crop rotation are not effective (Chamorro et al., 2015).

The biology of the fungus is not yet completely known. The teleomorph stage of the fungus is unknown (Premamalini et al., 2012). On Potato-Dextrose-Agar (PDA) *Rhizoctonia bataticola* develops only the microsclerotial form. Two different isolates of the pathogen are said to be compatible if they are capable of creating hyphal anastomoses (hypha bridges) between each other (Sharma et al., 2016). Anastomosis means the fusion of one fungal hyphal cell with another, genetically compatible hyphal cell (de Novais et al., 2017). Through the anastomoses their genetic material migrates from one isolate to another, resulting in genetic recombination (Mihail and Taylor, 1995). If two isolates are incompatible, barrier zones or blocking zones develop between the isolates (Csöndes, 2009). Genetic variability of the fungus is very important. If one strain of the fungus transfers its genetic material into another strain which has already adapted to certain environmental conditions new and more adaptive strains can evolve (Sharma et al., 2016). The results of compatibility tests of *M. phaseolina* isolates were referred to only once in Hungary (Csöndes, 2011). The main goal of this study was investigation of genetic variability and vegetative compatibility-incompatibility of *Macrophomina phaseolina* among different strains. Csöndes (2011) investigated the mycelial compatibility of 53 *Macrophomina phaseolina* isolates in Hungary. Csöndes (2011) described that the most of the isolates were compatible, only 24 pairs of all the possible paired combinations showed incompatible relationships. These results suggest that the same or very similar genotypes may spread over long distances, probably through the transportation of seeds or crops contaminated with microsclerotia (Csöndes, 2011). In the present study we aimed also to identify the type of microclerotia of studied pathogen.

Materials and methods

Mycelial compatibility of *M. phaseolina* isolates from different locations was investigated in the laboratory. In total the 30 samples collected were tested to examine their compatibility. The sunflower samples examined were collected from 2019 to 2020 in Hungary (29 isolates) and Slovakia (1 isolate).

All of the collected sunflower stem samples were disinfected with 75% ethanol for 4–5 minutes and rinsed with sterile water. Little pieces of infected stems were placed on 15 ml PDA media in 90 mm Petri dishes to culture and were incubated for 7 days at 30 °C under dark conditions. Growing cultures were transferred on new PDA. Mycelial discs with a diameter of 0.5 mm were cut out from all pure cultures. Three different mycelial discs were placed in each Petri dish on PDA. In the first Petri dish samples No. 2 and 3 were added next to sample No. 1; in the second Petri dish samples No. 4 and 5 were added next to strain No. 1; and this process was continued until all strains had been evaluated. All Petri dishes were incubated for 7 days under dark conditions. After incubation the relationship between two isolates was examined. If two strains were compatible the mycelia grew into each other and produced hyphal bridges (Fig. 1) which are visible clearly by microscope. The relationship between two isolates was considered incompatible when a blocking zone or barrier was formed at the hyphal contact site.

The types of microsclerotia (Fig. 2) were investigated according to Haig's characterization (Haig, 1929). Haig (1929) described 3 groups of *M. phaseolina* microsclerotia. According to the characterization, the size of Group 'A' is 1 mm, the average size of Group 'B' is 200 µm and the size of Group 'C' is about 120 µm. Randomly selected 50 microsclerotia from each pure culture were measured under a microscope, in total diameter of 1,500 microsclerotia were measured. The results for each pure culture were averaged.

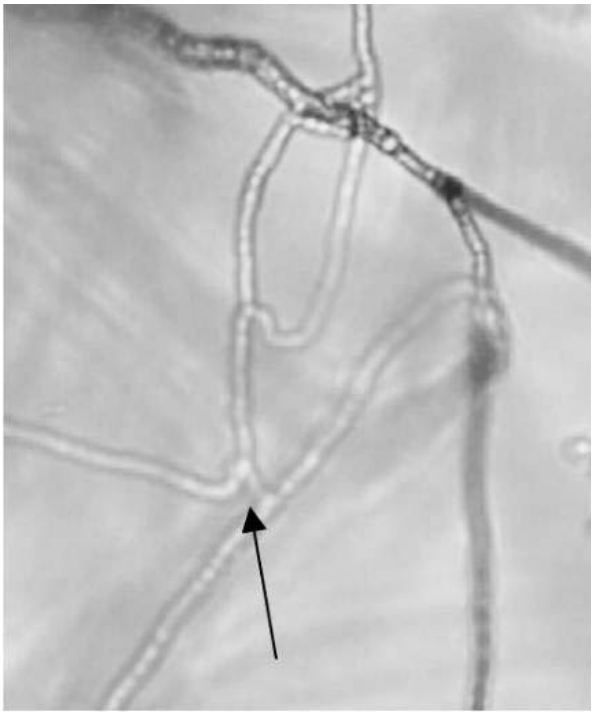

Fig. 1. Anastomosis between two compatible isolates.

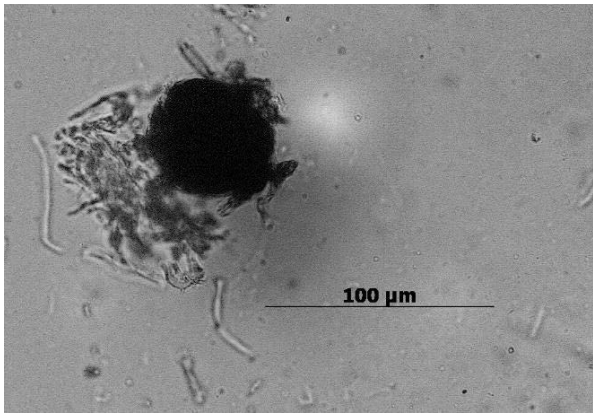

Fig. 2. *Macrophomina phaseolina* microsclerotium.

Table 1. Incompatible pairs of tested isolates

Incompatible pairs		
3 – 9	6 – 19	8 – 27
6 – 8	6 – 26	12 – 13
6 – 17	8 – 12	23 – 26
6 – 18	8 – 21	23 – 29

Results and discussion

A total of 465 pairing tests were made with 30 isolates. The results of our examination showed incompatibility in 12 pairings (Table 1).

Most often, isolate number 6 was incompatible with a total of 5 other isolates. Isolate number 8 was incompatible with 4 other isolates. Isolate number 6 originates from Berettyóújfalu and isolate number 8 originates from Hajdúböszörmény (Fig. 3).

The places of origin of the incompatible isolates were located far away from each other, more than 120 km in most cases, except for Berettyóújfalu (No. 6 in Fig. 3) and Hajdúböszörmény (No. 8 in Fig. 3) (70 km), Penyige (No. 23 in Fig. 3) and Hajdúdorog (No. 29 in Fig. 3) (80 km), as well as Berettyóújfalu (No. 6 in Fig. 3) and Körösszakál (No. 19 in Fig. 3) (30 km). Results of previous similar studies have shown that there is incompatibility mostly between isolates far from each other. It should be noted, that 2 compatible isolates were collected close to each other (5 km) from the fields of Komádi village (Komádi-1 and Komádi-2). There was one sample originating from Slovakia No. 24 in Fig. 3 from Sturovo) among the examined samples, which was compatible with all Hungarian samples.

Result of the current study is important because the teleomorph stage of the fungus is not yet known (Premamalini et al., 2012), and genetic recombination can occur between two compatible strains only by hyphal bridges. Based on our research, it can be said that most of the isolates collected close to each other were compatible, while Csöndes (2009) found that slightly different genotypes occur in areas close to each other. According to our results most of the isolates whose origins are far apart were also compatible with each other. This result verifies the theory that the microsclerotia of the pathogen are able to travel long distances. In the future, we will try to collect samples also from neighboring countries to demonstrate our theory.

In Hungary, mycelial compatibility of *M. phaseolina* was investigated only once (Csöndes, 2011). In this study the mycelial compatibility of 53 isolates collected from sunflower, maize and soybean in different regions of Hungary was determined (Csöndes, 2011). Similarly most isolates were compatible, only 24 pairs of all the possible paired combinations showed incompatible relationships, even geographically distant isolates were found to be compatible (Csöndes, 2011). In previous studies by Csöndes (2011), strains from Serbia were tested and all Serbian strains were found to be compatible with every Hungarian sample. The results confirmed that the fungus probably spread to Hungary from Serbia. The Slovakian isolate was compatible with all Hungarian isolates, so it is possible that the fungus spread to Slovakia from Hungary; although this theory requires further investigation.

Genetic variability of *Rhizoctonia bataticola* is assumed to be due to mutation, hyphal fusion and mitotic recombination (Sharma et al., 2016). Between incompatible isolates, there is a genetic difference and this requires further molecular biological examinations as well. The genetic variability of the pathogen can also cause additional difficulties for practical plant protection. Mycelial compatibility tests were done with another pathogen of sunflowers, *Sclerotinia sclerotiorum* fungus in Hungary by Zándoki (2005), who identified isolates originating from a greater distance from each other generally as compatible, while there is more often incompatibility between those which are geographically close to each other. In this study

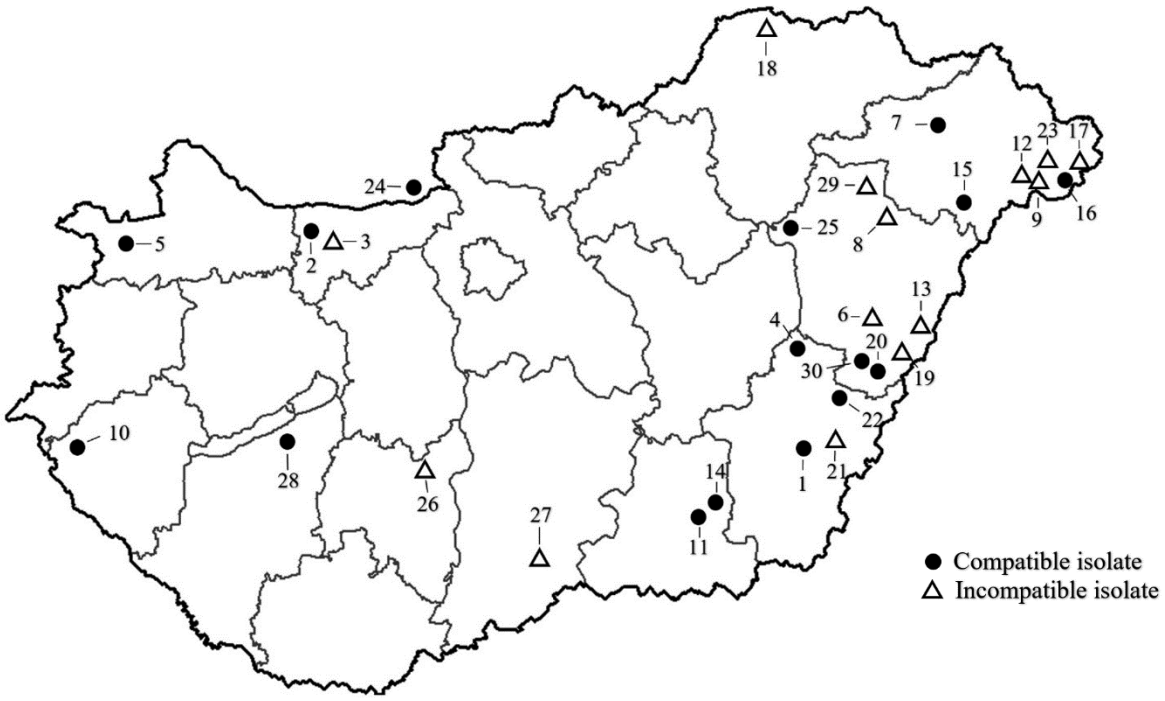

Fig. 3. Locations of sampling in different regions of Hungary and Slovakia.1, Békéscsaba; 2, Bana; 3, Bábolna; 4, Bucsa; 5, Röjtökmuzsaj; 6, Berettyóújfalu; 7, Buj; 8, Hajdúböszörmény; 9, Csenger; 10, Szombathely; 11, Székkutas; 12, Kocsord; 13, Kismarja; 14, Kakasszék; 15, Nyírmihálydi; 16, Nagygéc; 17, Zajta; 18, Rakaca; 19, Körösszakál; 20, Komádi 1st sample; 21, Doboz; 22, Vésztő; 23, Penyige; 24, Sturovo; 25, Egyek; 26, Bikács; 27, Mélykút; 28, Karád; 29, Hajdúdorog; 30, Komádi 2nd sample.

we have also pointed out that incompatible relationships can occur between strains originating from sites that are a greater distance from each other.

The diameters of microsclerotia from the studied pure cultures were in each case between 74 and 182 µm. The average diameter of the measured microsclerotia was 128 µm. It was found that in the case of the Hungarian samples all microsclerotia belonged to Group 'C'. In previous study, Békési (1970) identified in Hungary similarly the same type of microsclerotia, Group 'C'.

In summary, we can say that *M. phaseolina* is one of the very important invasive polyphagous pathogens for the future of crop production in southern areas of the globe as well as in Europe (Tarankata et al., 2003). Due to global warming, the fungus spreads easily to northern areas, and its genetic variation also helps in the migration of the pathogen.

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