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Investigation of the mycelial compatibility of *Macrophomina phaseolina* in the Carpathian Basin

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SUMMARY

Macrophomina phaseolina is a globally widespread fungal pathogen. The fungus has a very wide range of hosts. Under optimal conditions, M. phaseolina can cause serious damage tothe host plants. In this study, the mycelial compatibility of different M. phaseolina isolates was investigated. From 2019 to 2021, 12 sunflower samples were collected from different regions of the Carpathian Basin, 9 samples from Hungary, 1 sample from Austria, and 2 samples from Slovakia. The genetic variability of the pathogen is a critical problem in plant protection. Two compatible pathogen strains can easily exchange their genetic material for each other, which can lead to the development of resistance. All collected samples (12) were tested to examine their compatibility. Isolates from all tested samples were paired with isolates from all other samples thus, a total of 66 pairings were made. During the examinations of mycelial compatibility, only 20 pairs of all possible pairings were found to be incompatible, and all others (46) were found to be compatible.

Keywords: *Macrophomina phaseolina, mycelial compatibility, anastomoses, hyphal bridge, barrier zone*

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid. [synanamorph: *Rhizoctonia bataticola* (Taubenh.) E. J. is a dangerous fungal pathogen. It has several host plants, such as sunflower, corn, sorghum, soybean, and other important economic crops (Meyer et al., 1972). The fungus is prevalent in arid, tropical, and subtropical climates, though it can easily adapt to colder and rainy weather as well (Raut and Bhombe, 1984). *M. phaseolina* was reported in colder countries such as Belgium (Hunt, 1952), United Kingdom (Scholefield and Griffin, 1979), Switzerland (De Barros, 1985), Czech Republic (Šárová et al., 2003) and Slovakia (Bokor, 2007).

The infectious capacity of *M. phaseolina* is influenced by environmental factors such as temperature or rainy weather. Several authors have investigated the effect of temperature on the growth of *M. phaseolina*. According to Parmar et al. (2018), the ideal temperature for mycelial growth and the development of microsclerotia is 28–32 C. *M.* phaseolina survives in soil, seed, and stubble (Khan, 2007). According to Singh and Singh (1982) and Santos et al. (1984) in most cases, the fungus is transmitted by seeds.

The main symptoms are ashy spots on the stems and visible black microsclerotia in the pith (Purkayastha et al., 2006). Infected host plants lose their vitality, wither, and are characterized by premature death (Csöndes, 2009). Around the blooming period, when the temperature is high, and there is water deficiency symptoms are dramatic and progressive on many crops (Blanco-Lopéz and Jiménez-Diáz, 1983). Under dry and stressful conditions, the fungus can cause serious damage to the sunflowers, which can reach up to 100% (Damtea and Ojiewo, 2016).

The pathogen has two anamorph stages. Since the sexual stage of this fungus is unknown, parasexual recombination has an important role in reproduction (Premamalini et al., 2012). *Macrophomina phaseolina* is in the pycnidia form, while *Rhizoctonia bataticola* is in the microsclerotia form, which can produce small, black microsclerotia. The microsclerotia form is the main problem in the practice. The pycnidia stage was detected only once in Hungary, on the stem of beans (Vajna and Békési, 2009).

Two different isolates are compatible if they can create hyphal anastomoses (hypha bridges) between each other. Two compatible isolates can replace their genetic material through their anastomoses, to maintain the genetic variability, which may also lead to the development of possible fungicide resistance. If two isolate are incompatible, barrier zones or blocking zones appear between them. (Csüllög and Tarcali, 2020).

So far, in Hungary, results of compatibility tests of *M. phaseolina* were published by Csöndes (2011) and Csüllög and Tarcali (2020). Csöndes examined 50 Hungarian, 2 Spanish and 1 Serbian isolates. Most examined strains were found to be compatible with each other. It found an incompatible connection in only 24 cases of all the pairing options. According to the study performed by Csüllög and Tarcali, incompatibilities occurred in only 12 cases out of the total pairing possibilities (465) of the 29 examined Hungarian and the 1 Slovakian isolates. Marvasti and Banihashemi created the first Mycelial Compatibility Groups (MCGs) of Macrophomina phaseolina in Iran. Marvasti and Banihasehemi (2019) investigated a total of 112 isolates of *M. phaseolina* from different hosts in different regions of Iran. A total of 33 different MCGs were identified. The research shows the existence of high genetic diversity among the fungal isolates. Marvasti and Banihashemi (2019) reported that there

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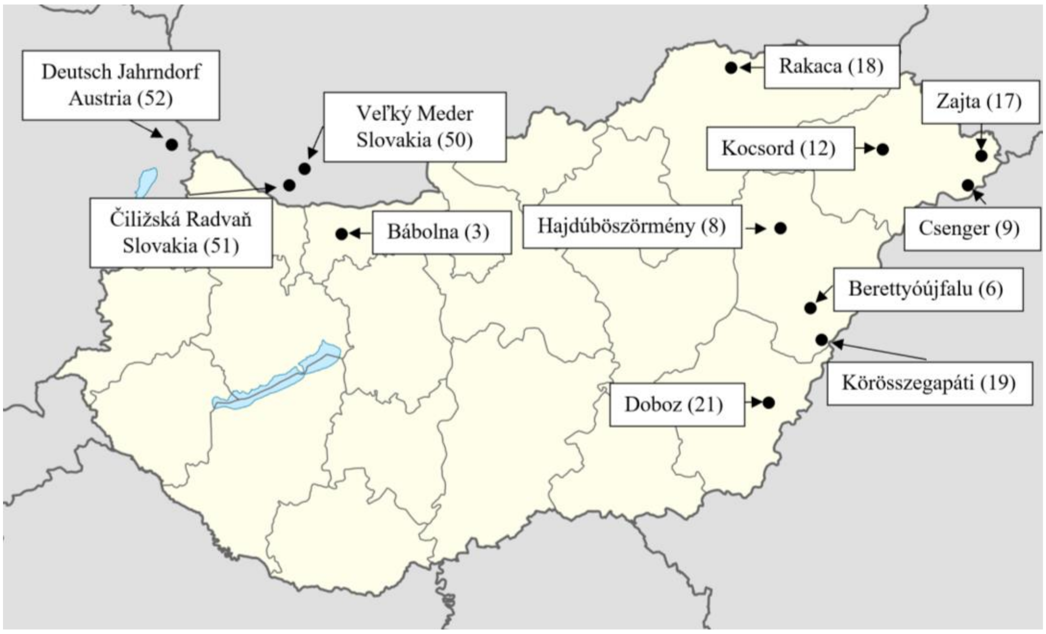
was no relationship between mycelial compatibility grouping, geographic distributions and host origins of the isolates. The main target of this study was to investigate the vegetative compatibility of *Macrophomina phaseolina* among different Carpathian basin strains.

MATERIALS AND METHODS

12 sunflower samples were collected from 2019 to 2021 from different locations in the Carpathian Basin (*Figure 1*). Most samples were found in Hungary but some samples are of Austrian and Slovakian origin.

The collected *M. phaseolina* samples were examined in the laboratory. First, pure cultures of the pathogen were made on Potato Dextrose Agar (PDA) media. After this, the pure cultures were incubated for 7 days under dark conditions at 30 C. Then, PDA media was poured into 90 mm Petri dishes for the compatibility tests. These isolates were sterilized under UV light for 20 minutes. Compatibility tests of 3 isolates were made in one Petri dish. These cultures in the Petri dishes were also incubated for 7 days under dark conditions at 30 C. After the incubation, connections between the tested strains were identified with a microscope. If hypha anastomoses were developed between two different isolates, they were compatible. If barrier zones were observable between two samples in the Petri dish, they were incompatible with each other. This method following by Csöndes (2009).

Figure 1: **Locations of the collection of *Macrophomina phaseolina* isolates**



RESULTS AND DISCUSSION

A total of 66 pairing tests were made of 12 isolates. In the case of 20 pairing incompatibility was detected. The other 46 pairings were compatible with each other (*Table 1*). The sample which was collected in Austria was compatible with 3 Hungarian isolates and was incompatible with 4 Hungarian isolates. The Austrian sample was compatible with one of the Slovakian samples (Veľký Meder) and incompatible with the other Slovakian sample (Čiližská Radvaň). The Slovakian sample from Čiližská Radvaň was compatible with 7 Hungarian samples and incompatible with 2 Hungarian samples. The Slovakian sample from Veľký Meder was compatible with 4 Hungarian samples and incompatible with 5 Hungarian isolates.

Based on the results, the isolates with the most incompatible relationships are the Berettyóújfalu (6) isolate of Hungarian origin, the two isolates from Slovakia (50; 51) and the Deutsch Jahrndorf (52) isolate. The Berettyóújfalu (6) isolate was found to be incompatible with another pathogen strain in 6 compatibility tests, the Veľký Meder isolates (50) in 5 cases, the Čiližská Radvaň isolates (51) in 5 cases and the Deutsch Jahrndorf isolates (52) in 6 cases.

During our investigation we found out that the collected samples even if they are close to each other can show incompatibility (for example Čiližská Radvaň and Veľký Meder), this means that several genotypes occur almost in the same area.

While isolates that were collected geographically far away from each other (for example Veľký Meder from Slovakia and Deutsch Jahrndorf from Austria) have shown compatibility in some cases. This result may suggest that identical or very similar genotypes can be easily spread with the help of microsclerotia.

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Table 1: **Incompatible pairs of tested isolates**

Incompatible pairs				
Bábolna (3) – Csenger (9)	Berettyóújfalu (6) – Čiližská Radvaň (51)	Kocsord (12) – Veľký Meder (50)	Rakaca (18) – Veľký Meder (50)	Körösszegapáti (19) – Deutsch Jahrndorf (52)
Berettyóújfalu (6) – Hajdúböszörmény (8)	Hajdúböszörmény (8) – Doboz (21)	Kocsord (12) – Čiližská Radvaň (51)	Rakaca (18) – Čiližská Radvaň (51)	Doboz (21) – Veľký Meder (50)
Berettyóújfalu (6) – Zajta (17)	Hajdúböszörmény (8) – Deutsch Jahrndorf (52)	Kocsord (12) – Deutsch Jahrndorf (52)	Rakaca (18) – Deutsch Jahrndorf (52)	Veľký Meder (50) – Čiližská Radvaň (51)
Berettyóújfalu (6) – Rakaca (18)	Csenger (9) – Deutsch Jahrndorf (52)	Zajta (17) – Veľký Meder (50)	Körösszegapáti (19) - Veľký Meder (50)	Čiližská Radvaň (51) – Deutsch Jahrndorf (52)

Remarks: The location names in the table are the origins of the isolates, and the numbers in parentheses are the serial numbers of the isolates obtained in the experiment.

CONCLUSIONS

The present research was based on the selection of isolates showing incompatible relationships from previous studies (Csüllög and Tarcali, 2020). 9 Hungarian isolates were selected and tested with 3 Carpathian Basin isolates of foreign origin.

In our experiment, it was found that geographically distant isolates may have different genotypes. This fact was previously reported by Csöndes (2009). However, Csöndes (2009) found that there were also incompatibilities between geographically close isolates. This shows that many genotypes can be present simultaneously in a small area. Csüllög and Tarcali (2020) also confirmed the results found by Csöndes. There was an incompatible connection between the Bábolna (3) and Csenger (9) isolates, which are more than 400 km apart. However, there was also an incompatible connection between the Berettyóújfalu (6) isolate and the Körösszegapáti (19) isolate, where the distance is only 20 km.

Unfortunately, due to globalization high amount of inocula can be imported with tropical fruits, different kinds of vegetables or even the soil. Because of climate change, non-native fungi will be able to colonize in new natural habitats causing trouble and difficulties in plant protection. According to the research of Magyar et al. (2021), in most cases, the imported tropical fruits can be infected with *Aspergillus*, *Fusarium*, *Penicillium Chaetomium*, *Mucor*, *Paecilomyces* and *Scopulariopsis*. These facts are not surprising at all, because Santini et al. (2018) have determined that the main pathway of plant-pathogen introduction in Europe, is the import of plants.

One possible spread of *M. phaseolina* microsclerotia is through the import of seeds. If infected seeds are sown in an area may appear new *M. phaseolina* strains. The compatibility conditions of the new strains are not known, so seed testing becomes particularly important.

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