

Genetic Diversity of *Macrophomina phaseolina* Isolates from Different Regions and Host Plants

Sonja Tančić, Boško Dedić, Aleksandra Dimitrijević, Ivana Imerovski, Nenad Dušanić, Dragana Miladinović

Institute of Field and Vegetable Crops Novi Sad, Maksima Gorkog 30, 21 000 Novi Sad, Serbia
e-mail: sonja.tancic@ifvcns.ns.ac.rs

ABSTRACT

• *Macrophomina phaseolina* (Tassi) Goid. is a soil borne pathogen with a wide host range which causes disease on more than 500 cultivated and wild plant species. The absence of host specificity and its geographic distribution suggest that the pathogen is heterogeneous. Current research was undertaken to elucidate the genetic diversity of *M. phaseolina* isolates obtained from different hosts and geographical locations.

• Seventeen *M. phaseolina* isolates were analysed for their genetic polymorphisms by Random Amplified Polymorphic DNA (RAPD). *M. phaseolina* isolates were collected from nine locations in Serbia, two locations in Bulgaria and one location in Turkey. Tested isolates were mainly recovered from sunflower, but also maize, soybean and flax.

• RAPD analysis confirmed previous observations that there is considerable variability within this pathogen depending on the host and location. RAPD has been reported as a useful tool for detecting genetic variation in *M. phaseolina* population as it was confirmed in our research.

• So far it was little known about genetic diversity of *M. phaseolina* from Serbia, and these results are first report about genetic variability of Serbian *M. phaseolina* isolates. Furthermore, phylogenetic relations of *M. phaseolina* isolates from Serbia with the isolates from other regions are also determined.

Key words: genetic diversity, *Macrophomina phaseolina*, RAPD, sunflower

INTRODUCTION

Macrophomina phaseolina (Tassi) Goid. is a soil borne pathogen with a wide host range which causes disease (charcoal rot) on more than 500 cultivated and wild plant species, including sunflower, maize, soybean, sugar beet, peanut, common bean, flax, sesame etc. The qualitative differences in *M. phaseolina* are related to the fungus host specificity, and it varies from crop to crop. Furthermore, difference in isolates aggressiveness depending on host of origin, as well as on cross-pathogenicity was proved (Suriachandraselvan et al., 2005; Su et al., 2001). This fungus infects the plant root in a seedling stage and if the invaded plant survives, the fungus slowly moves to the above-ground parts of the plant. First visible symptoms of sunflower charcoal rot such as root and basal stem rot, grey black stem discoloration and progressive wilting, cannot be noticed till a flowering stage, which makes this disease difficult for chemical control.

Development of charcoal rot symptoms is favoured by hot and dry conditions and host maturity. Temperatures near 30°C and dry conditions make this pathogen prevalent in regions with arid subtropical and tropical climates such as Pakistan (Khan, 2007), China (Xiaojian, 1988) and India (Suriachandraselvan et al., 2006) where yield losses caused by this fungus can reach even 90% of yield. Charcoal rot of sunflower can also be registered in moderate climates when high temperature and dry conditions occurs such as in Russia (Yakutkin, 2001), USA (Gulya et al., 2002), as well as in some European countries - Spain (Jiménez-Díaz et al., 1983), Portugal (de Barros, 1985), Italy (Manici et al., 1992), Hungary (Csöndes et al., 2011), Slovakia (Bokor, 2007), Czech Republic (Veverka et al., 2008), Romania (Ionită et al., 1996), Bulgaria (Alexandrov, 1999) and Serbia (Aćimović, 1998). Generally, it is estimated that charcoal rot affects the crop throughout the world reducing seed yields by 20-36% (Jimenez-Díaz et al., 1983).

Host specificity and geographic distribution over which it is found suggests that *M. phaseolina* is quite heterogeneous. The fungus host specific behaviour and a high degree of variation in its morphological, cultural and pathological properties, even when it is isolated from different parts of the same plant, makes this pathogen difficult to control. Therefore, understanding the genetics of host and pathogen as well as host-pathogen relationship in disease development is important for successful breeding for disease resistance.

Current research was undertaken to elucidate the genetic diversity of *M. phaseolina* isolates obtained from different hosts (sunflower, maize, soybean, flax) and geographical locations (Serbia, Bulgaria, Turkey).

MATERIAL AND METHODS

Collection of isolates. Samples of *M. phaseolina* were obtained from naturally infected plants from different regions of Serbia, Bulgaria and Turkey. Collections were made during period of 2009-2011. Origin of tested isolates, as well as host plants they were isolated from, is given in Table 1.

Table 1. Tested isolates data – isolate code, origin, host plant and year of isolation.

No	Isolate Code	Site, Region (Country) of Origin	Host Plant	Year of Isolation
1.	H-T19	Edirne, Trakia region (Turkey)	Sunflower	2011
2.	H-T120	Edirne, Trakia region (Turkey)	Sunflower	2011
3.	H-B72	Dobrudja, North region (Bulgaria)	Sunflower	2010
4.	H-B73	Burgas, South region (Bulgaria)	Sunflower	2010
5.	H-S11	Bačka Topola, Vojvodina region (Serbia)	Sunflower	2009
6.	H-S12	Kula, Vojvodina region (Serbia)	Sunflower	2009
7.	H-S13	Pančevo, Vojvodina region (Serbia)	Sunflower	2009
8.	H-S14	Bajmok, Vojvodina region (Serbia)	Sunflower	2009
9.	H-S15	Zrenjanin, Vojvodina region (Serbia)	Sunflower	2009
10.	H-S16	Kuštilj, Vojvodina region (Serbia)	Sunflower	2009
11.	H-S19	Deliblato, Vojvodina region (Serbia)	Sunflower	2009
12.	H-S23	Bezdan, Vojvodina region (Serbia)	Sunflower	2009
13.	H-S37	Rimski Šančevi, Vojvodina region (Serbia)	Sunflower	2009
14.	M-S38	Rimski Šančevi, Vojvodina region (Serbia)	Maize	2009
15.	M-S39	Rimski Šančevi, Vojvodina region (Serbia)	Maize	2009
16.	F-S40	Rimski Šančevi, Vojvodina region (Serbia)	Flax	2009
17.	S-S70	Rimski Šančevi, Vojvodina region (Serbia)	Soybean	2010

Isolation of the fungus. Samples of stems showing typical symptoms of *M. phaseolina* infection were collected. Pieces of infected tissue were placed on PDA medium and after 7 days colonies with morphology and growth characteristics of *M. phaseolina* were selected for further purification. Suspensions of microsclerotia were dispersed on water-agar plates, and after 24h pure culture of each isolate were made by hyphal tip transfer technique. These purified isolates were used for genetic analyses.

DNA isolation. DNA of *M. phaseolina* was isolated directly from a 7-days old mycelium grown at 29°C on PDA in the dark. Samples were frozen in liquid nitrogen and grounded to fine powder with a mortar and pestle. For further DNA extraction CTAB protocol was used (Permingeat et al., 1998).

RAPD analyses. PCR amplification was done according to protocol of Nagl et al. (2011), in 25 µl reaction volume containing 2.5 µl of reaction buffer (Fermentas); 1.5 mM MgCl₂, 0.2 mM dNTP; 0.5 µM primers, 2 unit Taq polymerase (Fermentas) and approx. 100 ng DNA was used. Amplifications were carried out in a Mastercycler ep gradient S termocycler (Eppendorf) with the following program: denaturation at 94 °C for 4 min followed by 40 cycles of 94 °C for 2 min, 36 °C for 1 min and 72 °C for 2 min, with final extension at 72 °C for 10 min. PCR products were separated on 2% agarose gels containing ethidium bromide and visualized under UV light. DNA polymorphism was evaluated in reactions with five RAPD primers: 1. UBC 159 (5'-GAGCCCGTAG-3'), 2. UBC 272 (5'-AGCGGGCCA-3'), 3. UBC 292 (5'-AAA CAG CCCG-3'), 4. UBC 353 (5'-TGGGCTCGCT-3') and 5. UBC 358 (5'-GGTCAGGCC-3').

Data analysis. DNA bands which could be confidently scored for presence or absence were included in the analyses. Each fragment that was amplified using RAPD primers was treated as binary unit character and scored "0" for absence and "1" for presence. An unweighted pair group arithmetic mean method (UPGMA) cluster analysis was performed, using average linkage method. The dendrogram was constructed by using STATISTICA 7.0 (StatSoft).

RESULTS AND DISCUSSION

RAPD has been reported as a useful tool for detecting genetic variation in *M. phaseolina* population (Mayék-Pérez et al., 2001; Su et al., 2001; Jana et al., 2003; Babu et al., 2010; Csöndes et al., 2011), and in our research the RAPD analyses were carried out with five primers and 17 isolates of *M. phaseolina* originating from different geographical regions and host plants. In total, 128 fragments using five primers were generated. All fragments generated by UBC 159, UBC 292 and UBC 353 were polymorphic, while 2 fragments out of 17 generated by UBC 358, and 7 fragments out of 27 generated by UBC 272 were monomorphic. The primer which generated the largest number of fragments (31) and the most polymorphism among isolates was UBC 159 (Fig. 1).

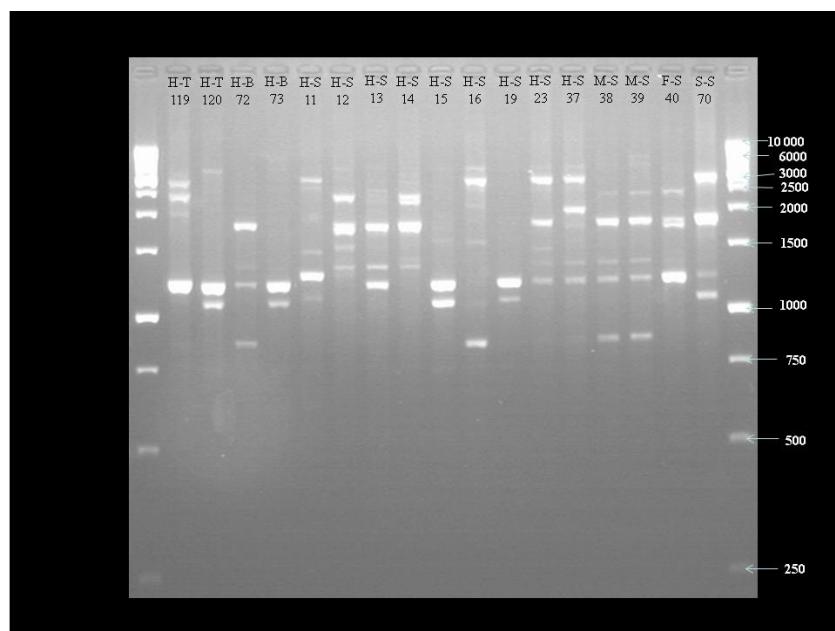


Figure 1. Agarose gel electrophoresis pattern of the RAPD profiles of *M. phaseolina* isolates with primer UBC 159

According to RAPD analyses and dendrogram (Fig. 2) obtained by UPGMA method it is clearly visible that isolate H-T119 originating from sunflower in Turkey was genetically similar with 2 Serbian isolates – H-S70 and H-S16 from soybean and sunflower, respectively. Another Turkish isolate H-T120 was genetically distant from H-T119 which clearly shows variability of *M. phaseolina* isolates originating from the same location in Turkey, but it was similar to H-B73 isolate from South region of Bulgaria. This similarity might be due to geographical proximity of these two locations and it can be assumed that the same or similar haplotypes were spread over a long distances. This was also concluded by Csöndes et al. (2011) for two Serbian and Hungarian *M. phaseolina* isolates which were found to be similar despite their different geographical origin. Certain similarity of two Serbian isolates (H-S15 and H-S19), both from South-East part of Vojvodina, with above mentioned isolates from Turkey and South Bulgaria, can be also noticed in this research (Fig. 2) which may indicate that those isolates might have been evolved from a common ancestor and that overlapping of pathogen populations occur in these countries. Genetic variability among isolates from South and North region of Bulgaria was also recorded, as well as variability *M. phaseolina* sunflower originating isolates from different locations in Serbia. Concerning a host plant from which tested isolates were isolated, only grouping of maize originated isolates (M-S38 and M-S39) can be noticed as well as their similarity with isolate from flax (F-S40). On contrary to this, isolate from soybean (S-S70) was significantly different from those from maize and flax (Fig. 2).

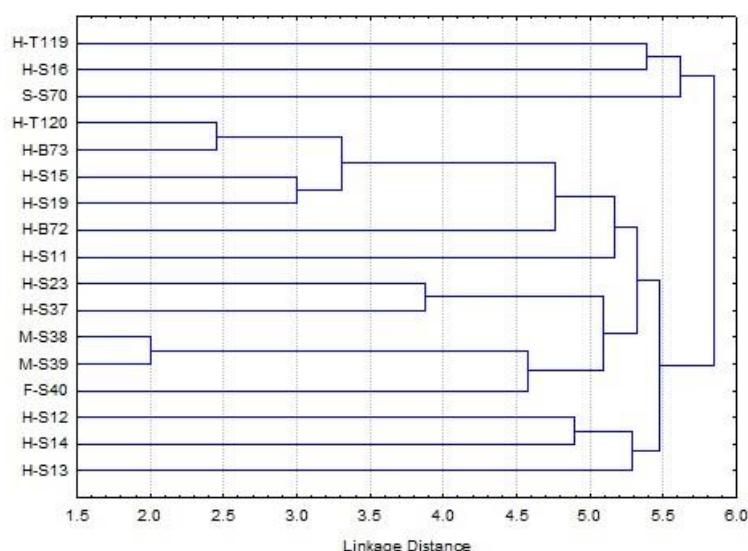


Figure 2. Dendrogram constructed by UPGMA clustering method with Euclidean distances for the data obtained in RAPD assay of 17 *M. phaseolina* isolates

So far scientist did molecular research of *M. phaseolina* world-wide, and there were some tendencies of the isolates to form groups related to geographical origin (Mayék-Pérez et al., 2001; Jana et al., 2003; Su et al., 2001), but also the opposite – isolates grouping irrespective of host (Babu et al., 2010) and geographical origin (Csöndes et al., 2011). It was little known about genetic diversity of *M. phaseolina* from Serbia, and these results are the first report about genetic variability of Serbian *M. phaseolina* isolates. According to the results of this research, isolates cannot be clearly differentiated according to their geographical origin, but phylogenetic relations of *M. phaseolina* isolates from Serbia with the Bulgarian and Turkish isolates are determined, as well as genetic variability of those isolates. High degree of genetic variation in the pathogen makes development of resistant sunflower hybrids very difficult, and therefore knowledge about the genetic variation in host and pathogen are necessary for successful breeding. Hence, greater number of primers and *M. phaseolina* isolates originating from neighbouring countries as well as Serbia will be included in the further research.

AKNOWLEDGMENTS

This research is part of the project TR 31025 supported by Ministry of Education and Science of Republic of Serbia. The authors are especially thankful to dr Valentina Entcheva (Dobrudzha Agricultural

Institute, Dobrudzha, Bulgaria) for providing the fungal isolates from Bulgaria, as well as dr Yalcin Kaya and dr Goksal Evci (Trakya Agricultural Research Institute, Edirne, Turkey) and dr Gülden Hazarhun (May-Agro Seed Corp., Yildirim, Turkey) for providing the fungal isolates from Turkey.

REFERENCES

- Aćimović, M., 1998. Charcoal root and stem rot. p. 544-567. In: Sunflower diseases. Scientific Institute of Field and Vegetable Crops, Novi Sad, Serbia (In Serbian).
- Alexandrov, V. 1999. Incidence of charcoal rot of sunflower caused by *Sclerotium bataticola* Taub. in Bulgaria. Bulg. J. Agric. Sci. 5: 867-890.
- Babu, B.K., Reddy, S.S., Yadav, M.K., Sukumar, M., Mishra, V., Saxena, A.K. and D.K. Arora. 2010. Genetic diversity of *Macrophomina phaseolina* isolates from certain agro-climatic regions of India by using RAPD markers. Indian J. Microbiol. 50 (2): 199-204.
- Csöndes, I., Cseh, A., Taller, J., and P. Poczai. 2011. Genetic Diversity and Effect of Temperature and Ph on the Growth of *Macrophomina phaseolina* Isolates from Sunflower Fields in Hungary. Mol. Biol. Rep. Available at <http://www.ncbi.nlm.nih.gov/pubmed/21695429> (Verified 21. November 2011).
- De Barros, L.M. 1985. Disease Complex (*Fusarium oxysporum* and *Macrophomina phaseolina*) Responsible for Sunflower Wilt in Portugal. p. 445-448. In: Proc. 11th Int. Sunfl. Conf., Mar del Plata, Argentina. Int. Sunfl. Assoc., Parise, France.
- Ionita, A., Iliescu, H. and S. Kupferberg. 1996. *Macrophomina phaseolina* – One of the Main Pathogens of Sunflower Crop in Romania. p. 718-723. In: Proc. 14th Int. Sunfl. Conf., Beijing, China, Int. Sunfl. Assoc., Parise, France.
- Jana, T., Sharma, T.R., Prasad, R.D. and D.K. Arora. 2003. Molecular characterization of *Macrophomina phaseolina* and *Fusarium* species by a single primer RAPD technique. Microbiol. Res. 158: 249-257.
- Jiménez-Díaz, R.M., Blanco-López, M.A. and W.E. Sackston. 1983. Incidence and distribution of charcoal rot of sunflower caused by *Macrophomina phaseolina* in Spain. Plant Disease. 63: 1033-1036.
- Manici, L.M., Cerato, C. And F. Caputo. 1992. Pathogenic and Biological Variability of *Macrophomina phaseolina* (Tassi) Goid. Isolates in Different Areas of Sunflower Cultivation in Italy. p. 779-784. In: Proc. 13th Int. Sunfl. Conf., Pise, Italy. Int. Sunfl. Assoc., Parise, France.
- Meyék-Pérez, N., López-Castañeda, C., González-Chavira, M., García-Espinosa, R., Acosta-Gallegos, J., Martínez de la Vega, O. And J. Simpson. 2001. Variability of Mexican Isolates of *Macrophomina phaseolina* based on pathogenesis and AFLP genotype. Physiol. Mol. Plant Path. 59: 257-264.
- Nagl, N., Taški-Ajduković, K., Barać, G., Baburski, A., Seccareccia, I., Milić, D., Katić, S. 2011. Estimation of the Genetic Diversity in Tetraploid Alfalfa Populations Based on RAPD Markers for Breeding Purposes. Int. J. Mol. Sci. 12: 5449-5460.
- Permingeat, H.R., Romagnoli, M.V. and R.H. Vallejos. 1998. A Simple Method for Isolating High Yield and Quality DNA from Cotton (*Gossypium hirsutum* L.) Leaves. Plant Mol. Biol. Rep. 16: 1-6.
- Su, G., Suh, S.O., Schneider, W. and J.S. Russian. 2001. Host Specialization in the Charcoal Rot Fungus, *Macrophomina phaseolina*. Phytopath. 91: 120-126.
- Suriachandraselvan, M., Aiyyanthan, K.E.A. and R. Vimala. 2005. Host range and cross inoculation studies on *Macrophomina phaseolina* from sunflower. Madras. Agric. Journal. 92 (4-6): 238-240.
- Suriandraselvan, M., Salalrajan, F., and K.E.A. Aiyyanathan. 2006. Relationship between morphological variations and virulence in the isolates of *Macrophomina phaseolina* causing charcoal rot of sunflower. Madras. Agric. J. 93 (1-6): 63-67.
- Yakutkin, V.I. (2001). Sunflower diseases in Russia and their control. Plant protection and caratine. 10: 26-29. (In Russian)
- Xiaojian, L., Liu, L.I., Baidnun, O. and Z. Derong. 1988. Geographical distribution of sunflower diseases in China. p. 16-20. In: Proc. 12th Int. Sunfl. Conf. Novi Sad, Serbia.