Pure Appl. Biol., 7(4): 1309-1315, December, 2018
http://dx.doi.org/10.19045/bspab.2018.700152

Research Article

Isolation and characterization of *Macrophomina phaseolina* isolates prevailing in Sindh, Pakistan

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Citation
Khadim Hussain Wagan, Muhammad Ibrahim Khaskheli, Jamal-U-Ddin Hajano and Abdul Ghani Lanjar. Isolation and characterization of *Macrophomina phaseolina* isolates prevailing in Sindh, Pakistan. Pure and Applied Biology. Vol. 7, Issue 4, pp 1309-1315. http://dx.doi.org/10.19045/bspab.2018.700152

Received: 05/07/2018 Revised: 24/08/2018 Accepted: 02/09/2018 Online First: 07/09/2018

Abstract

Macrophomina phaseolina (Tassi) Goid is economically important fungal pathogen with wide host range. In this study we found morphological and phenotypical variation among isolates of the fungus infecting sunflower crop in Sindh province of Pakistan. Thirty two *M. phaseolina* isolates were obtained from infected plant samples of sunflower collected from different ten districts viz., Badin, Thatta, Hyderabad, Tando Muhammad Khan, Shaheed Benazirabad, Mirpurkhas, Sanghar, Dadu, Sukkur and Khairpur. Significant variation in characteristics was noticed among collected isolates. They were usually black, blackish-gray, grayish-black and gray in colony color. Generally three growths pattern; dense, feathery and restricted were seem. Among them dense was most common (50.0%) followed by feathery (34.36%) and restricted (15.64%). Maximum colony growth (90.0%) and minimum (65.0%) was recorded after 7 days of inoculation on medium. Feathery growth was generally fast and restricted growth was slow. Maximum average linear colony growth (13 mm) and minimum (9 mm) growth was noted. Isolates showed significantly varied sizes of microsclerotia. Maximum sclerotia size (124.0 µm) was obtained from fields of Badin while, minimum size of microsclerotia (83.0 µm) from sunflower fields of Sukkur. Microsclerotia from dense growth pattern were black and big in size and small microsclerotia from gray growth pattern.

Keywords: Charcoal rot; Isolates and Characterization; *Macrophomina phaseolina;* Sunflower

Introduction

Macrophomina phaseolina (Tassi) Goid. which is dominant fungal pathogen of sunflower and is polyphagous causing disease to many other crop species. Importance of *M. phaseolina* rot is increasing with time because of attack on root, stem and fruit of more than 500 plant species worldwide, especially in regions where temperature is high **[1, 2]**. Up to 90% losses due to this fungus are reported in sunflower under favorable conditions for infection **[3]**. The fungus belongs to Phylum: *Ascomycota*, class: *Botryosphaeriales*, order: *Botryosphaeriales* and family: *Botryosphaeriaceae*. *M. phaseolina* isolates

are differing in various morphological and other aspects like mycelium colour, microsclerotia distribution, pycnidia formation and chlorate phenotypes [4]. Iqbal and Mukhtar [5] reported 65 isolates of *M. phaseolina* from Punjab and Khyber Pakhtunkhwa (KPK) provinces of Pakistan which are varying in morphological characteristics and pathogenic nature to infect mungbeen crop. Ashraf *et al*. [6] conducted such study in Punjab province to differentiate morphologically and virulent potential of 24 isolates of this fungus from maize crop which are significantly changed in the geographic regions. Furthermore, morphological and virulent variation among isolates of *M. phaseolina* collected from mash is examined by Riaz *et al*. [7]. Better understanding variability within pathogen population for traits, survival and fitness in different agro-ecological regions, definitely may support in design improved management strategies [8]. Hence, this study was planned to characterize *M. phaseolina* isolates prevailing in sunflower fields of Sindh province of Pakistan.

Materials and methods

Collection of samples

Plant samples showing charcoal rot symptoms and soil samples near root zone of infected plants were collected from various farmer's fields and research stations at sunflower growing districts of Sindh. All samples were brought to the laboratory for further studies.

Isolation and identification of *Macrophomina phaseolina* isolates

Plant sample

After surface washing of plant samples under tap water; small pieces (0.5 cm) from symptomatic stem and roots were cut and disinfected with 1% sodium hypochlorite for 2 minutes and then rinsed three times in sterilized distilled water. Infected small pieces were placed on Petri plates (5pieces per plate) containing potato dextrose agar (PDA) medium. All Petri plates were incubated at 30 ^{o}C for 7 days. Further, pure culture of the fungus was maintained by hyphal tip method. Isolates of *M. phaseolina* were named in abbreviate along with host plant and where numbered in numerical digits starting from 1 to 32.

Morphological characterization of *Macrophomina phaseolina* isolates

Colony growth

To calculate growth rate of isolates, 5 mm disc from active growth of fungus was taken and placed at center of 90 mm diameter Petri plate containing sterilized PDA medium. All Petri plates were incubated at 30 ^{o}C for 7 days. Each treatment was replicated five times. Observation on growth speed was taken by measuring diameter of Petri plates in mm per day and at the time of experimental termination. Furthermore observations on colony color and growth pattern were recorded for each isolates.

Sclerotia size

Slides were prepared from 7 days old pure culture of each isolates and examined under microscope ocular micrometer. Length and width of ten randomly selected sclerotia was measured in µm and were presented as average of both dimension. Additionally shape of sclerotium was noticed for each isolate.

Statistical analysis

Data regarding radial growth, average growth (mm/day) and sclerotia size (µm) was statistically analysis for determining LSD at $\alpha = 0.005$ using STATISTIX v. 8.1 software (Analytical Software).

Results

Remarkable variation in morphological and phenotypical characteristics was noticed in purified cultures of *M. phaseolina*. Thirty two *M. phaseolina* isolates were obtained from infected plant samples of sunflower collected from different ten districts of Sindh (Table 1 & Figure 1). They were usually black, blackish-gray, grayish-black and gray

in colony color (Table 2). Generally three growth patterns; dense, feathery and restricted were seen (Table 2). Among them dense was most common (50.0%) followed by feathery (34.36%) and restricted (15.64%) (Table 2). Maximum colony growth (90.0%) and minimum (65.0%) was recorded after 7 days of inoculation on PDA medium. Feathery growth was generally faster than restricted type growth (Table 2). Average linear colony growth per/day ranged from 9-13 mm, which significantly differ with each other (Table 2). Sclerotium size also significantly varied among the isolates. Isolates showed significantly varied sizes of microsclerotia. MPS16 isolate collected from fields of Badin showed maximum sclerotium size (124.0 µm) while, minimum size of sclerotium (83.0 µm) was measured in MPS29 obtained from sunflower fields of Sukkur (Table 2). Microsclerotia from dense growth pattern were black and big in size and small microsclerotia from gray growth pattern.

Table 1. Isolates of *Macrophomina phaseolina* obtained from different sunflower farmer's fields of Sindh

District	Isolates
Badin	MPS12, MPS13, MPS14, MPS15, MPS16, MPS17
Thatta	MPS18, MPS19, MPS20
Hyderabad	MPS1, MPS2, MPS3, MPS4, MPS5
Tando Muhammad Khan	MPS9, MPS10, MPS11
Shaheed Benazirabad	MPS21, MPS22, MPS23
Mirpurkhas	MPS6, MPS7, MPS8
Sanghar	MPS24, MPS25, MPS26
Dadu	MPS31, MPS32
Sukkur	MPS29, MPS30
Khairpur	MPS27, MPS28

Discussion

Collected cultures of *M. phaseolina* significantly varied in growth pattern, colour frequency and shape as well size. Thirty two isolates were obtained from infected plant samples of sunflower collected from different ten districts of Sindh, during this study. They were usually black, blackish-gray, grayish-black and gray in colony color. Generally three growth patterns; dense, feathery and restricted were seem. Among them dense was most common (50.0%) followed by feathery (34.36%) and restricted (15.64%). Maximum colony growth (90.0%) and minimum (65.0%) was recorded after 7 days of inoculation on medium. Feathery growth was generally fast and restricted growth was slow. Saleh *et al.* **[9]** and Atiq *et al*. **[10]** also reported three types of colony growth (dense, feathery and restricted) patterns of *M. phaseolina* isolates when grown on media. Aboshosha, *et al.* **[11]** also reported similar findings about growth pattern of *M. phaseolina*.

Maximum average linear colony growth per day (13mm) and minimum (9mm) growth was noted. Isolates showed significantly varied sizes of microsclerotia. Karunanithi *et al*. **[12]** described cultural and pathogenic variation of isolates of *M. phaseolina* into three groups. Isolates were significantly varied in ability to produce sclerotia. Maximum mycelial growth (90 mm) on PDA was recorded by I_{14} and the minimum by I_3 (72.5 mm). Linhai, *et al*. **[13]** observed rich variations in morphological characteristics, including density of aerial mycelia, sclerotia quantity, sclerotium size and growth speed of colony of *M. phaseolina*.

Maximum sclerotia size (124.0µm) was obtained from fields of Badin while, minimum size of microsclerotia (83.0µm) from sunflower fields of Sukkur. Microsclerotia from dense growth pattern were black and big in size and small microsclerotia from gray growth pattern. Suriachandraselvan and Seetharaman [14] reported that the most virulent isolate (MP8) produced highest mycelial growth (87.1mm) and abundant sclerotia in all the media within 48.8 h. while, Bradley and Rio [15] isolated black microsclerotia (76 ± 28 micro m in diameter) from vascular tissues of lower stems. The fungus was confirmed on the basis of colony colour, morphology and size of microsclerotia.

Table 2. Morphological characteristics of *Macrophomina phaseolina* isolates obtained from different districts of Sindh

Isolate	Colony color	Growth pattern	Radial growth (mm)	Average growth (mm/day)	Sclerotia size (µm)	Sclerotia shape
MPS1	Black	Dense	90a	13a	112j	Ovoid
MPS2	Blackish-gray	Feathery	90a	13a	107m	Irregular
MPS3	Black	Dense	87d	12ab	114h	Ovoid
MPS4	Blackish-gray	Dense	88c	13ab	113i	Ovoid
MPS5	Black	Dense	89b	13ab	117e	Round
MPS6	Grayish-black	Feathery	90a	13a	99p	Round
MPS7	Gray	Restricted	71f	10c	90t	Ovoid
MPS8	Grayish-black	Feathery	87d	12ab	100o	Irregular
MPS9	Blackish-gray	Feathery	90a	13a	108l	Oblong
MPS10	Black	Dense	89b	13ab	114h	Ovoid
MPS11	Black	Dense	89.2ab	13ab	115g	Ovoid
MPS12	Black	Dense	90a	13a	120c	Ovoid
MPS13	Black	Dense	88c	13ab	118d	Round
MPS14	Black	Dense	90a	13a	117e	Ovoid
MPS15	Blackish-gray	Dense	87d	12ab	122b	Ovoid
MPS16	Black	Dense	90a	13a	124a	Ovoid
MPS17	Black	Dense	90a	13a	116f	Round
MPS18	Black	Dense	87d	12ab	118d	Ovoid
MPS19	Blackish-gray	Dense	86e	12b	113i	Ovoid
MPS20	Blackish-gray	Feathery	90a	13a	107m	Ovoid
MPS21	Grayish-black	Feathery	90a	13a	92s	Oblong
MPS22	Grayish-black	Feathery	90a	13a	95q	Irregular
MPS23	Grayish-black	Feathery	89b	13ab	100o	Oblong
MPS24	Grayish-black	Dense	67i	10de	95q	Round
MPS25	Grayish-black	Feathery	90a	13a	93r	Oblong
MPS26	Grayish-black	Feathery	88c	13ab	88v	Irregular
MPS27	Grayish-black	Restricted	65j	9e	90t	Round
MPS28	Gray	Restricted	70g	10cd	86w	Round
MPS29	Gray	Restricted	69h	10cd	83x	Round
MPS30	Grayish-black	Restricted	71f	10c	89u	Round
MPS31	Blackish-gray	Dense	86e	12b	110k	Ovoid
MPS32	Grayish-black	Feathery	88c	13ab	104n	Irregular
LSD (alfa=0.5)			0.9021±0.456	0.4343± 0.2202	0.799±0.406	

Sclerotia size = length + width/2

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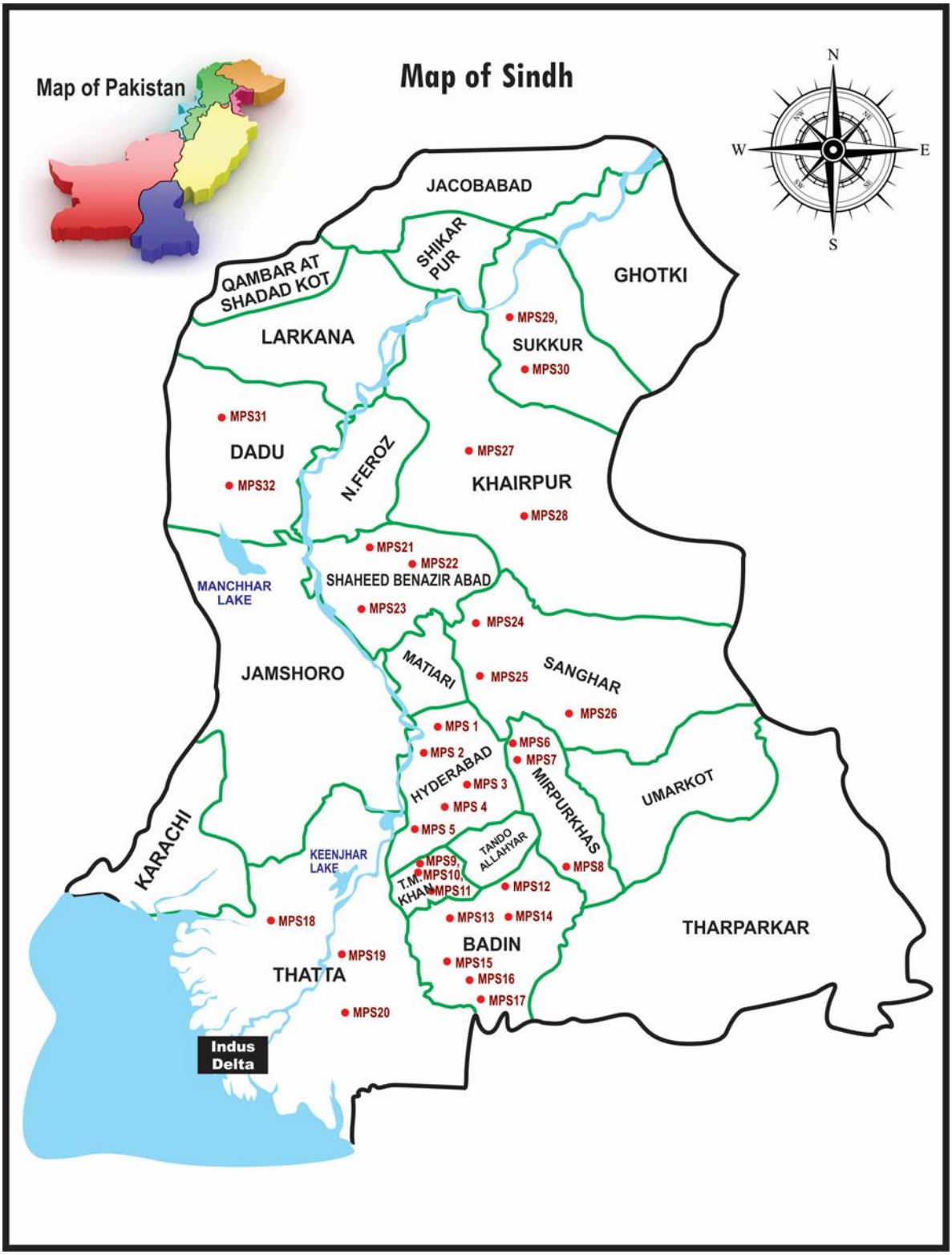

Figure 1. Prevlance of different *Macrophomina phaseolina* isolates in different districts of Sindh, Pakistan

Conclusion

Remarkable variation in morphological and phenotypical characteristics was noticed in purified cultures of *M. phaseolina*. Thirty two *M. phaseolina* isolates were obtained from infected plant samples of sunflower collected from ten different districts of Sindh. They were usually black, blackish-gray, grayish-black and gray in colony color. Generally three growth patterns; dense, feathery and restricted were seen and among them dense was most common. MPS16 isolate collected from fields of Badin showed maximum sclerotium size.

Authors' contributions

Conceived and designed the experiments: KH Wagan & MI Khaskheli, Performed the experiments: KH Wagan, Analyzed the data: J Hajano & AG Lanjar, Contributed reagents/ materials/ analysis tools: KH Wagan & J Hajano, Wrote the paper: KH Wagan.

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