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Relationship between morphological variations and virulence in the isolates of *Macrophomina phaseolina* causing charcoal rot of sunflower

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Abstract : The isolates of the pathogen significantly differed in their morphological characters. These produced cottony white with more fluffy to dull white and partially fluffy growth on PDA. The sclerotia were globose to irregular in shape and dark brown to black in colour with the size ranging from 70.0 x 58.1 μ to 168.8 x 130.0 μ. There was no correlation between the sclerotial size and virulence. But, the most virulent isolate (Mp8) produced the maximum sclerotial dry weight (1883 mg) as against the least (1046 mg) by Mp4. Cottony type mycelial growth and the sclerotial formations were negatively correlated.

Key words : Sunflower, charcoal rot, Macrophomina phaseolina morphological variations, virulence.

Introduction

Although only one species was recognised within the genus *Macrophomina,* great variability in the morphology was observed among the isolates occurring on different host plants. The isolates obtained from tobacco (Mungan and Yildiz, 1989) and different other host plants (Bansal *et al.,* 1990) differed in their colony colour and the mycelial growth pattern. Many earlier workers reported variations in the sclerotial size, shape and colour between isolates obtained from different parts of the same host (Jain *et al.,* 1973) and from different host species (Raut and Ingle, 1989). Variation in the virulence among the isolates of sunflower was reported by Jimenez-Diaz *et al.* (1983).

Materials and Methods

Collection of Macrophomina phaseolina isolates

Sunflower plants showing the typical charcoal rot symptoms incited by *Macrophomina phaseolina* (Tassi.) Goid. were collected from 25 conventional sunflower growing areas of Tamil Nadu. The pathogen was isolated from the roots of these infected plants by tissue segment method on potato dextrose agar (PDA) medium. Axenic culture of the pathogen was obtained by monosclerotial method and the purified cultures of the isolates were maintained on PDA slants for use in the studies.

Morphological characters of M. phaseolina isolates

The cultures of the isolates of *M. phaseolina* were multiplied on PDA medium in perti plates (90mm). The sterilized warm medium (20ml) was plated into each plate under aseptic condition and allowed to solidify. A five-day old actively growing eight mm PDA culture disc (cut using a sterilized cork borer) of the pathogen was placed onto the medium at the centre of the plate and incubated at room

Table 1. Growth and morphological characters of *M.phaseolina* isolates.

Isolates	Colony characters	Sclerotial dry weight (g)**	Sclerotial characters		
			Colour	Shape	Size (μ)*
Mp 1	Dull white, partially fluffy	1615	Dark Brown	Irregular	120.7 x 91.3
Mp 2	Grayish white, not fluffy, smooth	1329	Black	Globose	70.0 x 58.1
Mp 3	Dull white, partially fluffy	1387	Black	Globose	78.8 x 66.4
Mp 4	Cottony white, fluffy, fast growing	1046	Black	Irregular	168.8 x 130.0
Mp 5	Grayish white, not fluffy, smooth	1842	Dark Brown	Globose	89.5 x 75.2
Mp 7	Grayish white, not fluffy, smooth	1804	Black	Irregular	90.5 x 70.5
Mp 8	Grayish white, not fluffy	1883	Black	Irregular	112.4 x 84.1
Mp 9	Cottony white, fluffy	1757	Black	Irregular	98.2 x 77.2
Mp 10	Dull white, partially fluffy	1866	Black	Irregular	103.8 x 81.9
Mp 11	Cottony white, partially fluffy	1340	Black	Irregular	98.0 x75.5
Mp 12	Cottony white, partially fluffy	1569	Black	Globose	79.5 x 66.4
Mp 13	Grayish white, not fluffy, smooth	1737	Black	Globose	88.3 x 73.3
Mp 14	Grayish white, not fluffy, smooth	1738	Black	Irregular	94.0 x 77.0
Mp 15	Cottony white, fluffy	1737	Black	Globose	84.8 x 72.1
Mp 16	Grayish white not fluffy	1680	Black	Irregular	99.3 x 79.3
Mp 18	Grayish white, not fluffy	1661	Black	Irregular	90.5 x 74.0
Mp 19	Cottony white, more fluffy	1687	Dark Brown	Irregular	85.0 x 69.0
Mp 20	Dull white, partially fluffy	1611	Black	Irregular	73.3 x 58.7
Mp 21	Dull white, partially fluffy	1490	Black	Irregular	100.6 x 77.8
Mp 22	Grayish white, partially fluffy	1292	Dark Brown	Irregular	110.8 x 81.5
Mp 23	Grayish white, partially fluffy	1670	Dark Brown	Irregular	109.6 x 82.2
Mp 24	Grayish white, not fluffy	1853	Black	Globose	76.0 x 68.9
Mp 25	Cottony white, fluffy	1557	Dark Brown	Irregular	99.2 x 80.4
Mp 26	Grayish white, not fluffy	1841	Black	Globose	72.3 x 63.3
Mp 27	Grayish white, not fluffy	1744	Black	Globose	75.5 x 64.8

* Mean of 100 Sclerotia ; ** Mean of three replications

temperature (28 ± 2°C). The colony characters *viz.* colony colour and colony texture were observed 60 h after incubation.

Four PDA culture discs (8 mm) of the pathogen were removed seven days after plating individually; placed in a beaker containing 50 ml of sterile distilled water and stirred with a stirrer for five min to separate the sclerotia from the medium. The entire content was squeezed through cheesecloth; washed in several changes of distilled water and transferred to a glass vial containing 2.5 ml of 2.5 per cent ammonium sulphate. The sclerotia, which

Table 2. Virulence of *M.phaseolina* isolates on sunflower plants (artificial inoculation)*

Isolates	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling Vigour index	Disease incidence (%)
Mp 1	76.7 (61.14)	9.63	21.13	2359	85.0 (67.40)
Mp 2	81.7 (64.69)	10.77	23.33	2786	78.3 (62.48)
Mp 3	85.0 (67.40)	12.13	24.50	3117	61.7 (51.78)
Mp 4	85.0(67.21)	12.43	25.63	3236	66.7 (54.75)
Mp 5	81.7 (64.69)	11.37	24.30	2912	56.7 (48.87)
Mp 7	80.7 (63.55)	12.33	23.30	2848	68.3 (55.77)
Mp 8	71.7 (57.86)	8.60	18.07	1909	88.3 (70.12)
Mp 9	83.3 (65.95)	11.07	20.77	2650	61.7 (51.78)
Mp 10	73.3 (59.00)	8.80	18.50	2101	86.7 (68.66)
Mp 11	81.7(64.69)	11.47	20.37	2600	83.3 (65.95)
Mp 12	85.0 (67.40)	13.03	22.37	3013	80.0 (63.55)
Mp 13	83.3 (66.26)	11.47	20.60	2673	75.0 (60.00)
Mp 14	86.7 (65.95)	12.73	22.53	2943	70.0 (56.79)
Mp 15	83.3 (68.86)	13.07	24.40	3242	61.7 (51.78)
Mp 16	86.7 (68.66)	13.00	23.83	3194	70.0 (56.84)
Mp 18	85.0 (67.40)	14.30	24.53	3431	51.7 (45.96)
Mp 19	81.7 (65.19)	12.80	25.50	3131	48.3 (44.04)
Mp 20	85.0 (67.71)	13.63	25.37	3317	46.7 (43.09)
Mp 21	80.0 (63.55)	13.50	24.50	3035	48.3 (44.04)
Mp 22	83.3 (65.95)	13.07	25.03	3175	50.0 (45.00)
Mp 23	81.7 (65.00)	12.67	25.30	3105	58.3 (49.82)
Mp 24	78.3 (62.29)	11.67	20.77	2541	71.7 (57.86)
Mp 25	88.3 (70.12)	13.60	24.57	3371	41.7 (40.18)
Mp 26	78.3 (62.29)	11.17	21.27	2543	73.3 (58.93)
Mp 27	83.3 (65.95)	14.43	25.13	3295	38.3 (38.22)
Control	93.3 (75.24)	17.87	29.90	4457	0.0 (0.00)
CD (P=0.05)	6.17	1.75	1.60	346	4.81

* Mean of three replications
(Figures in parentheses are Arcsine transformed values)

floated after 10 min, were filtered through a Whatman No.l filter paper and rinsed with distilled water. The filter paper with these sclerotia was removed and the sclerotia were transferred to a petri plate and dried for two h in shade and the size of 100 sclerotia was measured under 10 x using an ocular micrometer in a calibrated microscope. The mean size (μ.) of the sclerotia was calculated (Dhingra and Sinclair, 1978). The shape and colour of the matured sclerotia were observed under l0 x in the microscope.

The sclerotial dry weight of each isolate was also estimated using Richard's broth. One hundred ml of the medium was distributed into each 250 ml Erlenmeyer flasks and sterilized by autoclaving at 1.4 kg cm^{-2} pressure for 15 min. and cooled. Each flask was separately inoculated with an actively growing

eight mm PDA culture disc of the respective isolate cut from a five - day old culture using a sterile cork borer. The flasks were incubated at room temperature (28 ± 2°C) for 30 days. Three replications were maintained for each isolate. The mycelial mat was filtered through a pre-weighed Whatman No.l filter paper and dried in the hot air oven at 60°C until a constant weight was obtained. The sclerotial dry weight was obtained by subtracting the weight of filter paper alone from the total weight.

Virulence of the isolates on sunflower plants

The sand maize inoculum of each of the isolate was thoroughly mixed with the sterilized (1.4 kg cm^{-2} pressure for 2 h) pot culture soil filled in earthen pots (30 cm) at 1:19 ratio, a week prior to sowing. Five surface sterilized seeds of the highly susceptible sunflower cv. CO 2 were sown in each pot. The pots were maintained in the glass house with uniform, regular and judicious watering. Three replications were maintained for each isolate. The observations on germination, root length, shoot length and seedling vigour were made 15 DAS and the disease incidence was recorded 75 DAS.

Results and Discussion

Isolates Mp4, Mp9, Mp11, Mpl2, Mpl5, Mpl9 and Mp25 produced cottony white and fluffier mycelial growth while others produced dull white or grayish white with partially fluffy growth (Table 1). Similar variations among the isolates were reported by Byadgi and Hegde (1985) from different host plants. The isolates obtained from tobacco (Mungan and Yildiz, 1989) and different other host plants (Bansal *et al.,* 1990) differed in their colony colour and the mycelial growth pattern. Manici *et al.,* (1992) observed that white to gray aerial mycelium was scarcely produced by some isolates of *M.phaseolina* of sunflower.

The sclerotia produced by the different isolates at maturity were globose to irregular and dark brown to black with the attached hyphae (Table 1). These observations corroborated with those of Byadgi and Hegde (1985) on diferent host plants. Srivastava and Dhawan (1983) reported that the sclerotia were black, round, oval and sometimes irregular in shape generally clothed with hyphae. Dhar and Sarbhoy (1992) observed spherical to oblong sclerotia.

In the present study the biggest (168.8 x 130.0 μ) sclerotia were produced by the isolate Mp4 followed by Mpl (120.7 x 91.3 μ) and Mp8 (112.4 x 84.1 μ) and the smallest (70.0 x 58.1 μ) by Mp2 (Table 1). Haigh (1930) studied many isolates of *M. phaseoli* from different hosts and divided these into three groups on the basis of the mean sclerotial size. Accordingly, all the isolates in the present study strictly confined to the group 'C' of Haigh with sclerotia measuring below 120 μm except those of Mp4, which were the biggest with a diameter of 168.8 x 130.0 μm. He also recorded the mean size of sclerotia of *M. phaseolina* from sunflower as 88-104 μm. Manici *et al.* (1992) reported that the sclerotial diameter of 64 sunflower isolates significantly varied from 72.8 to 127.8μ.

Of the 25, the isolate, Mp8 collected from Kovilpatti was the most aggressive (88.3% disease) while Mp27 of Sankarankoil was the least virulent (38.3%) (Table 2). Jimenez-Diaz *et al.,* (1983) also reported similar variations in the virulence of the pathogen-affecting sunflower. Earlier workers correlated the variation in virulence with the morphological characterstics of the isolates. Srivastava and Dhawan (1983) found that the virulence was directly related to sclerotial and pycnidial production. Monga and Raj (1994) concluded that bigger sclerotia

caused more disease incidence even at low inoculum level. Many earlier workers (Jain *et al.,* 1973; Manici *et al.,* 1992) also observed no significant correlation between the sclerotial size and pathogenicity of the isolates of *M. phaseolina* as observed in the present study. Probably the sclerotial formation reflected the nutritional level of a given isolate on its substrate as well as its genotypic relationship.

The weight (dry) of the sclerotia produced in Richard's broth by the isolates also significantly varied from 1883 by most virulent isolate (Mp8) to 1046 mg by Mp4. Hooda and Grover (1988) found that more pathogenic isolates produced more number of the sclerotia. Simosa and Delgado (1991) observed a negative correlation between the cottony mycelial growth and the sclerotial production. In the present study also the isolate Mp4 that produced cottony fluffy mycelial growth recorded the least sclerotial dry weight.

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