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Morphological variability among *Macrophomina phaseolina* associated with mulberry root rot in Tamil Nadu

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Abstract
Considerable variations were noted in radial mycelial growth, dry weight, sclerotial number, colony colour and texture among the *Macrophomina phaseolina* cultures isolated from mulberry root rot infected samples. Among 15 cultures, two cultures were fast growing (>80 mm), five cultures produce maximum mycelial dry weight (>55 mg) and five cultures produce abundant sclerotial number (>50). Cultures were categorized based on colour as: grayish black, black with white mycelium and black and on the basis of texture as: fluffy, partially fluffy and appressed growth.

Keywords: mulberry, root rot, radial growth, mycelial dry weight, sclerotial number, colony colour and texture

Introduction
Mulberry (*Morus* spp., Moraceae) is grown as a perennial crop for its foliage to feed silkworms (*Bombyx mori* L.). It is reported that about 60% of the cost of cocoon production goes towards mulberry leaf production [24, 2]. After the introduction of high yielding varieties, with large genetic diversity followed by intensive cultivation practices, mulberry has become susceptible to various diseases especially to root rot infection. This disease has developed into more alarming because of its epidemic character and propensity to kill the plant completely. *Macrophomina phaseolina* (Tassi.) Goid. was isolated from mulberry root samples collected from the charcoal rot infested gardens of South India [8]. *M. phaseolina* is a soil borne pathogenic fungus and produces cushion shaped black sclerotia [23]. Its prevalence could be enhanced by various physiological and ecological aspects such as low moisture contents, high temperature, and heat [11]. Disease severity is correlated with viable sclerotia present in the soil. Mulberry plants infected by charcoal rot at almost all growth stages. The disease caused red to brown lesions on roots and stems with the production of dark mycelia and black micro sclerotia. Ultimately the plant became defoliated and withered [1] and dried [6].
Adequate information on the variability among *M. phaseolina* infected mulberry is not available. Hence the present study examined the morphological variability among 15 cultures of *M. phaseolina* isolated from the mulberry root rot infestation, collected from the different Sericulture practicing areas of Tamil Nadu.

Materials and Methods
Collection of root rot samples
A total of 15 cultures of *M. phaseolina* were collected from 6 mulberry cultivating districts (Table 1) of Tamil Nadu. Root samples bearing micro sclerotia of the fungus and characteristic symptoms of root rot were collected from the farmers' fields. The diseased specimens were packed in paper bags and properly labeled, brought to the laboratory and stored at 4 ºC until processed for identification.

Isolation and purification of the pathogen
The root rot infected root samples were collected from the field and used for isolation of the pathogen. The pathogen was isolated from the infected root by tissue segment method [18] and purified by adopting hyphal tip method [22].

Determination of morphological variability
Morphological variability among 15 cultures of *M. phaseolina* was studied on the basis of the following parameters.

1. Radial growth

For studying variability in radial growth, the cultures were grown on Potato Dextrose Agar (PDA) [14]. Fifteen milliliters of autoclaved PDA was poured in 90 mm diameter Petri plates, allowed to solidify and inoculated in the centre with a 9 mm plug from the actively growing culture of the fungus. The plates were incubated at 28 ± 1 ºC for 5 days. Each culture was replicated 3 times. Observations were recorded up to 96 hours at 24 hours interval. After the stipulated period, the growth of each culture was measured in terms of colony diameter and their means were computed. On the basis of radial growth, after 72 hours the cultures were categorized as fast (>80 mm), medium (61-80 mm) and slow (<60 mm) growing [13].

2. Mycelial dry weight

Twenty ml of Potato Dextrose Broth (PDB) was poured into 150 ml conical flasks and were sterilized at 1.1 kg/cm^2 for 20 min. The flasks were inoculated with agar blocks (5 mm diam.) cut from actively growing margin of seven day old culture of *M. phaseolina.* Treatments were replicated thrice. The cultures were filtered through pre-weighed Whatman no. 1 filter paper after incubation for seven days at 30±1 °C. The mycelial mats were dried at 85 °C for 24 h to determine the mycelial dry weight yield. The actual weight of dry fungal mycelium was calculated using the following formula [3].

Weight of mycelium = (Weight of filter paper + Weight of Mycelium) – (Weight of filter paper)

Based on the mycelial dry weight the cultures were grouped into high, medium and low [4].

3. Sclerotial number

Sclerotial number in different cultures was calculated by observing the number of sclerotial bodies formed per microscopic field (10x × 10x) at 3 spots in a Petri plate were recorded. The cultures were classified based on sclerotial population as abundant (>50), average (40-50) and low (<40).

4. Colony texture and colour

After 7 days of inoculation the cultures were classified based on growth pattern [12] and colour [10].

Results

Table 1: *Macrophomina phaseolina* cultures isolated from mulberry plants from different districts of Tamil Nadu

S. No.	District	Location and Culture code
1.	Coimbatore	Annur (MP-7), Pethikuttai (MP-15), Thondamuthur (MP-10)
2.	Tirupur	Dharapuram (MP-14)
3.	Erode	Aapakoodal (MP-2), Ayyampalayam (MP-13), Gobichettipalayam (MP-8), Nallagoundanpalayam (MP-4), Nambiyur (MP-9), Othakuthirai (MP-5)
4.	Dharmapuri	Kaarimangalam (MP-3), Pappireddipatti (MP-12)
5.	Thoothukudi	Muthalur (MP-1), Soorankudi (MP-6),
6.	Tirunelveli	Aalankulam (MP-11)

T**able 2:** Morphological variations among different cultures *M. phaseolina*

Culture code	Dry weight of the mycelium (mg)	No. of sclerotia / microscopic field (10x × 10x)	Radial growth (mm)	Colony texture	Colony colour
MP-1	39 ^{f}	21.33 ^{k}	67.16^{cde}	Appressed	Grayish black
MP-2	32 ^{g}	34.66 ^{h}	54.06^{g}	Fluffy	Grayish black
MP-3	48 ^{d}	61.66 ^{a}	65.92^{de}	Fluffy	Grayish black
MP-4	55 ^{c}	49.00 ^{d}	68.60^{cd}	Fluffy	Black with white mycelium
MP-5	50 ^{d}	46.00 ^{e}	55.70^{g}	Partially fluffy	Black with white mycelium
MP-6	39 ^{f}	52.66 ^{c}	81.82^{a}	Partially fluffy	Grayish black
MP-7	50 ^{d}	54.66 ^{bc}	81.24^{a}	Appressed	Black
MP-8	57 ^{bc}	19.66 ^{k}	66.48^{de}	Appressed	Black
MP-9	40 ^{f}	39.66 ^{fg}	63.52^{e}	Appressed	Grayish black
MP-10	56 ^{bc}	37.00 ^{g}	59.70^{f}	Partially fluffy	Grayish black
MP-11	66 ^{a}	56.33 ^{b}	75.22$_{b}$	Partially fluffy	Black with white mycelium
MP-12	57 ^{bc}	40.33 ^{f}	66.98^{cde}	Fluffy	Black with white mycelium
MP-13	44 ^{e}	56.66 ^{b}	70.42^{c}	Appressed	Black with white mycelium
MP-14	45 ^{e}	31.33 ^{e}	74.96^{b}	Appressed	Black with white mycelium
MP-15	58 ^{b}	27.00 ^{j}	64.36^{e}	Appressed	Black with white mycelium
SED	1.3294	1.1837	1.7861		
CD (P=0.05)	2.7150 **	2.4175 **	3.6478**		

Table 3: Categorization of *M. phaseolina* on the basis of morphological parameters

a) Radial growth		
Category	**Number of culture**	**Culture code**
Fast growing (>80mm)	2	MP-6,7
Medium growing(60-80mm)	10	MP-1, 3, 4, 8, 9, 11, 12, 13, 14, 15
Slow growing (<60mm)	3	MP-2, 5, 10
Mycelial dry weight		
Heavy (>55 mg)	5	MP-8, 10, 11, 12, 15
Medium (40-55 mg)	7	MP-3, 4, 5,-7, 9, 13, 14
Low (<40 mg)	3	MP-1, 2, 6
Sclerotial number		
Abundant population (>50)	5	MP-3, 6, 7, 11, 13

Average population (40-50)	3	MP-4, 5, 12
Low population (<40)	7	MP-1, 2, 8, 9, 10, 14, 15
b) Colony colour		
Grayish black	6	MP-1, 2, 3, 6, 9, 10
Black with white mycelium	7	MP-4, 5, 11, 12, 13, 14, 15
Black	2	MP-7, 8
c) Colony texture		
Appressed	7	MP-1, 7, 8, 9, 13, 14, 15
Fluffy	4	MP-2, 3, 4, 12
Partially fluffy	4	MP-5, 6, 10, 11

Radial growth

The average radial growth of 15 cultures of *M. phaseolina* observed after 72 hours of incubation ranged from 54.06 to 81.82 mm (Table 2). Maximum colony diameter of 81.82 and 81.24 mm were observed in culture MP-6 and 7 proving to be the fast growing, while culture MP-2 and 5 showed the minimum radial growth of 54.06 and 55.70 mm and were rated as slow growing. Two cultures (MP-6 and 7) showed radial growth above 80 mm and were rated as fast growing while the growth of 3 cultures (MP-2,5 and 10) was found below 60 mm and were categorized as slow growing. The rest of the 10 cultures showed growth between 60 and 80 mm were classified as medium growing (Table 3).

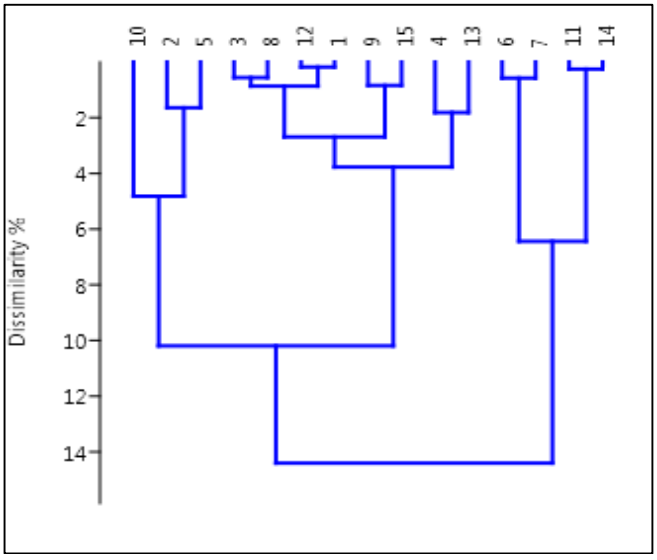

Fig 1: Dendrogram representation of cultures of *M. phaseolina* on the basis of radial growth

The dendrogram (cluster analysis - UPGMA) showed that four clusters were formed *viz*., first by MP- 10, 2 and 5 (<60 mm), the second by MP- 3, 8, 12, 1, 9, 15, 4 and 13 (60-70 mm), the third by MP- 6, 7, 11 and 14 (>70 mm). Based on colony diameter, the cultures included were grouped on the same branch with dissimilarity below 10%. The lowest dissimilarities were observed between cultures MP- 12 and 1, followed by MP- 11 and 14, and clustering between these pairs of cultures showed dissimilarities below 2%.

Mycelial dry weight

Maximum mycelial dry weight of 66 mg was produced by MP-11 and the least was MP-2 (32 mg) (Table 2). Out of 15 cultures, 5 (MP-8, 10, 11, 12 and 15) were grouped in the high range of mycelial weight (>55 mg), 3 (MP-1, 2 and 6) were ranked in the low range (< 40 mg) and 7 were in the range of (40-55 mg) and were ranked as medium (Table 3)

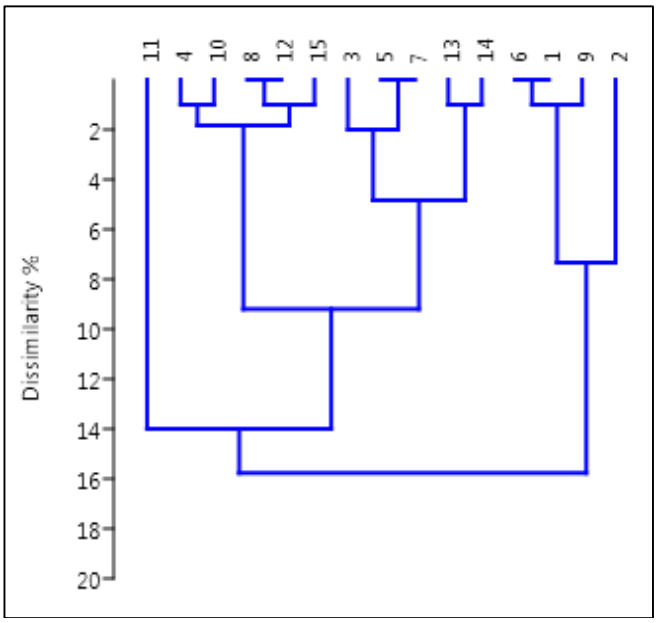

Fig 2: Dendrogram representation of cultures of *M. phaseolina* on the basis of mycelial dry weight

The dendrogram (cluster analysis - UPGMA) showed that four clusters were formed *viz*., first by MP - 4, 8, 10, 11, 12 and 15 (55-66 mg) the second by MP - 3, 5, 7, 13 and 14 (44-55 mg) the third by MP - 6, 1 and 9 (33-44 mg) and fourth by MP - 2 (<33 mg). Based on the dry weight of the mycelium, the cultures included were grouped on the same branch with a dissimilarity below 10%.

The lowest dissimilarities were observed between cultures MP-13 and 14, followed by MP-6 and 9, and clustering between these pairs of cultures showed dissimilarities below 2%. In 1st cluster MP- 8 and 12, in 2nd cluster MP- 5 and 7, 3rd cluster MP-6 and 1 were considered being identical because the dissimilarity was null.

Sclerotial number

The number of sclerotia varied from 19 to 62, among the cultures maximum sclerotial number was found in MP-3 (61.66) (Table 2). The 5 cultures MP-3, 6, 7, 11 and 13 were ranked as abundant sclerotial producer (*i.e.* >50). Less sclerotial number was found in MP-8 (19.66). The 7 cultures MP-1, 2, 8, 9, 10, 14 and 15 were come under the category of low sclerotial producer (*i.e.*<40) and the remaining 3 cultures MP-4, 5 and 12 were ranked as average sclerotial producer (*i.e.* 40-50) (Table 3).

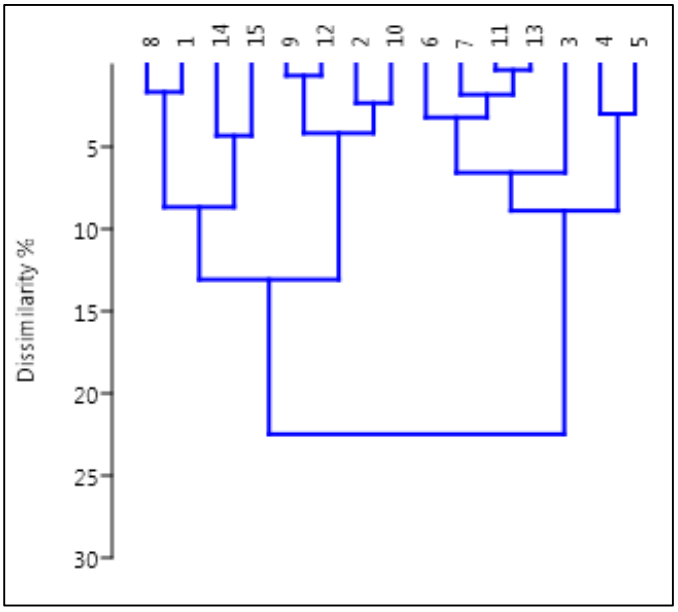

Fig 3: Dendrogram representation of *M. phaseolina* cultures on the basis of sclerotial number

The dendrogram (cluster analysis - UPGMA) showed that four clusters were formed based on sclerotial number *viz*., first by MP- 8, 1, 14 and 15 cultures (19-31), the second by MP- 9, 12, 2 and 10 (34-40), the third by MP- 6, 7, 11 and 13 (52-61) and the fourth by cultures MP- 4 and 5 (46-49). The cultures included were grouped on the same branch with dissimilarity below 10%. The lowest dissimilarities were observed between cultures MP- 11 and 13, followed by MP- 9 and 12, and clustering between these pairs of cultures showed dissimilarities below 5%.

Colony texture and colour

All cultures of *M. phaseolina* showed three phenotypes when grown on PDA medium *viz*., Appressed, Partially fluffy and Fluffy (Table 3). Seven cultures MP-1, 7, 8, 9, 13, 14 and 15 showed appressed growth. Four cultures MP-2, 3, 4 and 12 showed fluffy growth. The remaining four cultures MP-5, 6, 10 and 11 had partially fluffy appearance (Table 2).

Pigmentation varied from grayish black, black, and black with white mycelium (Table 3). Six cultures MP-1, 2, 3, 6, 9 and 10 produced grayish black mycelium, seven cultures MP-4, 5, 11, 12, 13, 14, and 15 produced black with white mycelium, two cultures MP-7 and 8 produced black mycelium (Table 2).

Discussion

Macrophomina phaseolina a soil-borne fungal pathogen, induces root rot in different crops including mulberry. In the present investigation, 15 cultures of *M. phaseolina* isolated from mulberry cultivated in different districts of Tamil Nadu exhibited variations in morphological parameters such as radial growth, mycelial dry weight, sclerotial number, colony colour and texture. The variations in morphology might be due to the difference in temperature, moisture, soil types and other edaphic factors of various districts of Tamil Nadu [20]. Morphological variability of *M. phaseolina* has also been reported by many workers in terms of growth, colour, sclerotial production and mycelial dry weight [13, 5, 16] which corroborated with the present study. However, the experiments conducted during the present study revealed that no relationship was found among the morphological characters of the isolated cultures. The earlier studies revealed that there were no relation between radial growth, size, weight of the mycelium and sclerotial number [13, 5].

The pathogenic fungus, *M. phaseolina* had a broad host range and exists in two asexual forms which maintain its survival better [11, 15, 9]. The variability existing in *M. phaseolina* was related to the phenomena of host specialization by several workers. Host specialization in maize and soybean on the basis of pathogenic, genetic and physiological differences were observed [21, 9] and this mechanism took long time to establish with a specific host. Due to heterogenic nature of *M. phaseolina*, categorization into distinct subgroups based upon pathogenicity and morphology could not take place [15]. Isolates having morphological similarity are not necessarily identical genetically, they might have some differences. The variable genetic pattern contributed to variation in morphology and pathogenesis which has been confirmed by using different molecular tools [19]. As the pathogen had no sexual phase, genetic diversity was produced either by fusion of vegetative cells or by parasexual recombination between nuclear gene [7]. In nature genetic variability improved the survival of fungus [17].

Conclusion

As there are no reports about the determination of morphological variability of *M. phaseolina* in mulberry, the present study for the first time has addressed it. The determination of variability among *M. phaseolina* isolates is fundamental to guide the development of appropriate strategies for root rot management. Hence, the results obtained in the present study will be useful in developing integrated strategies for the management of root rot problem.

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