Variability of *Macrophomina phaseolina* (Tassi.) Goid. the causal organism of root rot of sesame and its management

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DOI: [https://doi.org/10.22271/chemi.2020.v8.i2ab.9021](https://doi.org/10.22271/chemi.2020.v8.i2ab.9021)

Abstract

Ten isolates of *Macrophomina phaseolina* (Tassi) Goid. (sclerotial stage: *Rhizoctonia bataticola* (Taub) Butler) collected from different major sesame growing areas of Telangana, Karnataka and Andhra Pradesh were studied for their variability on morphological, cultural and pathogenic characteristics. The hyphal cell size varied from 13.75 x 3.75 μm (Mp 9) to 31.39 x 4.5 μm (Mp 5), size of sclerotia varied from 82.41 x 58.90 μm (Mp 6) to 135.18 x 101.88 μm (Mp 8). Based on shape of sclerotia the isolates were classified in to three groups. irregular, round and ovoid groups. The number of sclerotia per microscopic field at 10x varied from 14 (Mp 2) to 42 (Mp 7). There was no significant difference in the colony growth rate recorded among the isolates of *M. phaseolina* which varied from 7.66 to 8.65 mm, Isolates categorized into three groups viz., partially submerged, submerged, fluffy on the basis of colony texture and colony colour varied from black, grey and light grey colour. Based on the disease incidence isolates were categorized into two categories viz., virulent (2 isolates) and highly virulent (8 isolates).

Keywords: Variability, root rot, *Macrophomina phaseolina*, sclerotia

Introduction

The crop is cultivated in a wide range of atmospheres, extending from semi-arid tropics and subtropics to temperate areas of the world (Raikwar and Srivastava, 2013). India is the leading producer of sesame seeds in the world (FAO STAT, 2014) [8]. In India, sesame crop occupies an area of 1.77 Mha with production of 8.27 lakh tonnes and productivity of 426 kg ha⁻¹ (Indiastat, 2014-15) and in Telangana it is cultivated over an area of 0.28 lakh ha with the production of 0.09 lakh tonnes and productivity of 304 kg ha⁻¹ (Agricultural action plan 2015-16).

Sesame (*Sesamum indicum* L.) is under constant threat to many diseases viz., charcoal rot/stem rot/root rot, powdery mildew, leaf blight, wilt, leaf spot, stem blight, bacterial leaf spot and phylloxy. Among these root rot/stem rot caused by *Macrophomina phaseolina* (Tassi.) Goid (= *Rhizoctonia bataticola*) is the most important disease of sesame in India (Chattopadhyay and Sastry, 1998) [5]. It has become a potential threat for the profitable cultivation especially in the changing warm climate and intensive farming situations (Saharan *et al.*, 2005) [18]. In view of the importance of the crop and disease management, the present work is planned to study the culture characteristics, morphological and pathogenic variability among the isolates of *Macrophomina phaseolina*.

Materials and Methods

Morphological and cultural characterization of *M. phaseolina*

Ten isolates of the pathogen were selected for morphological and cultural characteristics (Table 1). Since the root rot disease of sesame is a major problem in Karnataka and Andhra Pradesh apart from Telangana so representative disease samples were collected from these two states also and pathogen was isolated. These isolates were further used in variability studies. The mycelial discs of 5 mm diameter were cut from the edge of a three days old culture and transferred aseptically to 90 mm Petridish containing 15ml PDA. These plates were incubated at 28±1 °C. Each treatment was replicated thrice.
**Table 1:** Isolates of *M. phaseolina* from different sesame growing areas

| Sl. No | Pathogen Isolated from | Isolate code |
|--------|------------------------|--------------|
| 1      | Polasa                 | Mp 1         |
| 2      | Laxmipur               | Mp 2         |
| 3      | Morthad                | Mp 3         |
| 4      | Renjarla               | Mp 4         |
| 5      | Bussapur               | Mp 5         |
| 6      | Babapur                | Mp 6         |
| 7      | IIOR farm, Rajendranagar, Hyderabad | Mp 7 |
| 8      | Narkhuda               | Mp 8         |
| 9      | Dharwar               | Mp 9         |
| 10     | Yelemanchili          | Mp 10        |

**Morphological variability**

The slides of various isolates were prepared from 10 days old culture for studying morphological characteristics viz., size of hyphal length, hyphal width, mycelial colour, size of sclerotia, number of sclerotia, colour of sclerotia and shape of sclerotia were recorded after 10 days of incubation by using Mycaps image analyser.

**Cultural variability**

The colonies of isolates were characterized for growth rate at 72 h after incubation. Seven days old cultures were used to record texture, colour of the colony and presence or absence of aerial mycelium.

**Pathogenic variability**

For assessing the pathogenic variability of *M. phaseolina* isolates sick pot soil method (Salunkhe et al. 2014) [23] was followed. The potting mixture was prepared thoroughly mixing clay loam soil, sand and farm yard manure at 1:1:1 ratio. The inoculum of each isolate of *M. phaseolina* collected from different locations were separately mixed at five per cent level (w/w) with the sterilized soil filled in 30 cm earthen pots ten days before sowing. Surface sterilized (using 0.1% mercuric chloride solution for 30 sec. followed by two washings in sterile water) sesame seeds were sown at 30 seeds per pot. Three replications were maintained in a completely randomized design and the sesame cultivar VRI-I was used in this study. The pots were maintained in glass house with regular, judicious and uniform watering. The data on germination was taken ten DAS and root rot incidence was recorded at 35 DAS, the per cent disease incidence was calculated and isolates are grouped as Least virulent, Virulent, Highly Virulent according to per cent disease incidence (Gupta et al. 2012) [9] as mentioned below.

| Isolate category   | Per cent mortality |
|--------------------|--------------------|
| Least virulent     | < 20 Per cent      |
| Virulent           | 21-50 Per cent     |
| Highly virulent    | > 51 Per cent      |

**Mass multiplication of the pathogen**

The inoculum of the test pathogen, *M. phaseolina* maintained on agar slants was further multiplied on sorghum grains. One hundred grams of sorghums eeds were washed thoroughly in tap water and soaked overnight in 250 ml conical flasks with addition of 20 ml of 4 per cent dextrose. The flasks were then autoclaved for 20 min at 15 lbs. After cooling the flasks at room temperature they were shaken well to separate the sterilized grains and were inoculated with 2-3 discs of 4 day old culture of *M. phaseolina* and incubated at 28 ± 1°C for seven days in BOD incubator. After seven days, the inoculum was mixed with sterilized soil in pots at five per cent level (w/w).

**Results and Discussion**

**Morphological variability in *M. phaseolina***

Variability in the cultural and morphological characteristics of ten isolates of *M. phaseolina* were studied on potato dextrose agar medium. The colony growth rate of the *M. phaseolina* isolates was measured at 72h after inoculation. The size of the hyphal cell and sclerotia was measured using Mycaps image analyzer software at 40x objective of the microscope. Observations on various cultural and morphological characteristics were recorded as described in material and methods and the results obtained are presented in Table 2 and Table 3.

**Hyphal cell**

The hyphal cell size varied from 13.75 x 3.75 μm (Mp 9) to 31.39 x 4.5 μm (Mp 5). Ratio between length and width of hyphal cell varied from 2.29 (Mp 8) to 6.97 (Mp 5). Significant variation was observed among the isolates regarding cell size, length and width of the hyphal cell. The isolates Mp 1 and Mp 3, Mp 4 and Mp 6 were on par with each other.

**Sclerotial size**

The data presented in Table 2, revealed that on the basis of microscopic observations the size of sclerotia varied from 82.41 x 58.90 μm (Mp 6) to 135.18 x 101.88 μm (Mp 8). The isolates were classified in to three groups, viz., small size (4000-5000 μm²) medium (5001-6000 μm²) and large size (more than 6000 μm²). Of the 10 isolates, one isolate (Mp 6) was categorized in small, 2 isolates (Mp 3 and Mp 10) were in medium and 8 isolates (Mp 1, Mp 2, Mp 4, Mp 5, Mp 7, Mp 8, Mp 9) in large sized sclerotia category based on classification given by Varma and Pathe (2013) [24] details are depicted in Table 2.1.

These findings are in confirmation with the work of Mandal et al. (1998) [14] who reported that the sclerotial size ranged between 66.14 - 128.25 um. Dhingra and Sinclair (1972) [6] observed variation in sclerotial size (173.88 - 188.33 x 51.09 - 155.90 um), Sobti and Sharma (1992) [21] reported sclerotial size of 60 - 165 x 57 - 114 um.

**Sclerotial shape**

The isolates were categorized into irregular, round and ovoid groups based on shape of sclerotia (fig.1). Irregular shaped sclerotia were observed in 3 isolates (Mp 7, Mp 8, Mp 9) while ovoid shaped sclerotia were observed in 6 isolates (Mp 1, Mp 2, Mp 4, Mp 5, Mp 6, Mp 10). Round shape of sclerotia was observed in one isolate (Mp 3). Details of categorization is mentioned in Table 2.2.

These results are in agreement with those findings reported by Mandal et al. (1998) [14], who observed irregular and round to elongate sclerotial shape. Gupta et al. (2012) [9] categorized the isolates into two groups viz., oblong shape with irregular edges and round with regular edges based on sclerotial morphology.
Fig 1: variability in sclerotial shape a: Oblong, b: Round c: Irregular shape.

Sclerotial intensity
The number of sclerotia per microscopic field when observed through 10x objective varied from 14 (Mp 2) to 42 (Mp 7). On the basis of number of sclerotia per microscopic field the isolates were grouped (Table 2) in to three groups viz., sparse (2 isolates), medium (2 isolates) and abundant (6 isolates). Details of categorization is depicted in Table 2.3. Similar results were obtained by Hooda and Grover (1982) [10] who reported that Hyderabad isolate produced highest number of sclerotia (180.3 sclerotia/ 9 mm. disc and 52/ microscopic 10 x field ) whereas Coimbatore isolate produced minimum number of sclerotia (169 sclerotia). Varma and Pathe (2013) [24] observed that the number of sclerotia ranged from 9.7 to 22.2 and 40 per cent of the isolates were having sparse number of sclerotia.

Cultural variability in M. phaseolina
Colony growth rate
There was no significant difference in the colony growth rate recorded among the isolates of M. phaseolina which varied from 7.66 to 8.65 mm (Table 3). Isolate Mp 7, showed highest colony growth (7.66 cm) while the least colony diameter was observed with the isolate Mp 3 (8.65 cm) at 72 hours after incubation. These results are in agreement with Varma and Pathe (2013) [24] who observed that in 22 isolates of R. bataticola isolates did not differ in the type of mycelial growth and growth rate on potato dextrose agar medium.

Colony colour
Based on visual observation on colony colour, the cultures were divided into three groups. Black, grey and light grey coloured colonies (fig.2). Three isolates produced black coloured colony. while three isolates had grey coloured colony and four isolates were found to have light grey colour colony (Table 3). Similar observations were also made by previous workers Shekhar et al. (2006) [20] on the basis of colony colour, divided seven isolates into four groups viz., grayish white, blackish grey, dark black in centre periphery cremish and cottony white colour. Mohanapriya et al., (2017) [22] also reported that all the ten isolates of the root rot pathogen M. phaseolina produced white, whitish grey, grey, black scanty to profusely aerial mycelial growth on Potato Dextrose Agar (PDA) medium.

Fig 2: Variability in colony colour: Black, b:Grey, c: Light grey colour

Colony texture
Isolates categorized into three groups viz., partially submerged, submerged, fluffy on the basis of colony texture (fig 3). Of the 10 isolates eight isolates (Mp 1, Mp 2, Mp 5, Mp 6, Mp 7, Mp 8, Mp 9, Mp 10) produced partially Submerged colony while one isolate (Mp 4) had Submerged texture and one isolate (Mp 3) had produced fluffy growth (Table 3). The colony texture of partially submerged was observed in Mp 1, Mp 2, Mp 5, Mp 6, Mp 7, Mp 8, Mp 9, Mp 10 isolates. Submerged texture was found in Mp 4, and fluffy growth in Mp 3 isolate. Details of grouping of isolates is given in Table 3.1. Similar observations were made by Varma and Pathe (2013) [24] who classified 22 isolates of R. bataticola on the basis of type of growth viz., fluffy and submerged, sixteen isolates were grouped in fluffy growth and six in sub merged group. Byadgi and Hedge (1985) [4] reported fluffy, submersed, dark brown mycelium and appressed light brown mycelial growth on different isolates of R. bataticola. Other workers have also reported the variation in type of growth of R. bataticola isolates (Sobti and Sharma, 1992, Ratnoo et al. 1997, Hooda and Grover, 1982) [21, 17, 10] grouped them in to 4-5 groups.
Pathogenic variability in *M. phaseolina*

Variation in pathogenicity was found among 10 isolates of the *M. phaseolina* when inoculated on sesame cultivar VRI-1. Eight isolates viz., Mp 1, Mp 2, Mp 4, Mp 5, Mp 6, Mp 7, Mp 8, and Mp 9 were found to be highly virulent while two isolates Mp 3, Mp 10 as virulent. All the isolates of *M. phaseolina* induced variable reaction on susceptible sesame cultivar VRI-1 in soil infestation technique (fig.4). Based on the disease incidence isolates were categorized into virulent (2 isolates) and highly virulent (8 isolates) as depicted in table 4.

Percent disease incidence varied from 44.35% to 73.33%. Accordingly eight isolates (Mp 1, Mp 2, Mp 4, Mp 5, Mp 6, Mp 7, Mp 8, and Mp 9) were categorized as highly virulent as the percent disease incidence was > 51 Per cent however, only two isolates (isolate Mp 3 and Mp 10) grouped as virulent isolates. Three groups i.e. virulent, intermediate and mild on the basis of pathogenicity has been reported by Byadgi and Hegde (1985) [41]. Other workers have also reported the differential reaction by the isolates of *R. bataticola* in chickpea (Ratnoo et al., 1997 and Monga and Sheo Raj, 1994) [17], and found considerable variation among the isolates.

Sobti and Sharma (1992) [21] also recorded 13 to 63 per cent root rot incidence of groundnut with different isolates of *R. bataticola*. Ratnoo et al. (1997) [17] reported the pathogenic variation in the isolates of *M. phaseolina* from different cowpea growing areas of Udaipur. The pathogenic variability of this fungus has been described in different host plants such as soybean and sunflower (Dhingra and Sinclair, 1978; Jimenez et al., 1983) [17], and variation in pathogenicity assumed to be due to mutation, hyphal fusion and mitotic recombination. The rapid growth or spread of the mycelia and the abundant occurrence of sclerotia due to conductive environmental conditions may also have caused variation (Jimenez et al., 1983) [17].

**Table 2: Variability in morphological characteristics of *Macrophomina phaseolina* isolates**

| Sl. No | Isolate Code | Length x Width (μm) | length : width ratio of hyphal cell | Length x Width (μm) | Area (μm²) | Intensity per 10x microscopic field | Shape | colour |
|-------|--------------|---------------------|-----------------------------------|---------------------|------------|-----------------------------------|-------|--------|
|       |              | Length | Width |                        | Length | Width |                          |       |        |
| 1     | Mp 1         | 24.47   | 5.50  | 4.44                    | 104.77 | 86.81 | 9095.08                      | 18    | Ovoid  |
| 2     | Mp 2         | 27.87   | 8.72  | 3.19                    | 96.61  | 74.42 | 7189.71                      | 14    | Black  |
| 3     | Mp 3         | 23.34   | 6.35  | 3.67                    | 97.38  | 59.80 | 5823.32                      | 15    | Round  |
| 4     | Mp 4         | 24.14   | 6.18  | 3.9                     | 96.48  | 80.79 | 7794.61                      | 23    | Ovoid  |
| 5     | Mp 5         | 31.39   | 4.5   | 6.97                    | 110.58 | 89.51 | 9898.01                      | 19    | Black  |
| 6     | Mp 6         | 24.49   | 5.87  | 4.17                    | 82.41  | 58.90 | 4853.95                      | 35    | Ovoid  |
| 7     | Mp 7         | 26.47   | 5.94  | 4.45                    | 91.13  | 71.56 | 6521.26                      | 42    | Irregular |
| 8     | Mp 8         | 21.46   | 9.34  | 2.29                    | 135.18 | 101.88 | 13772.1                      | 27    | Irregular |
| 9     | Mp 9         | 13.74   | 3.75  | 3.6                     | 92.96  | 74.44 | 6919.94                      | 29    | Irregular |
| 10    | Mp 10        | 27.53   | 4.62  | 5.9                     | 82.97  | 69.12 | 5734.88                      | 26    | Ovoid  |

C.D. | 0.86 | 0.51 | 0.34 | 0.58 | 0.23 | 57.74 | 0.37 | Black |
C. V. | 2.04 | 4.94 | 4.70 | 1.01 | 0.52 | 1.28 | 2.62 |        |
### Table 2.1: Grouping of isolates of *Macrophomina phaseolina* on the basis of sclerotial size Varma and Pathe (2013)

| Sl. No | Characters | Isolates |
|--------|------------|----------|
| 1      | Small (4000-5000 μm²) | Mp 6 |
| 2      | Medium (5001-6000 μm²) | Mp 3, Mp 10 |
| 3      | Large (more than 6000 μm²) | Mp 1, Mp 2, Mp 4, Mp 5, Mp 7, Mp 8, Mp 9, |

### Table 2.2: Grouping of isolates of *Macrophomina phaseolina* on the basis of intensity of the sclerotia

| Sl. No | Characters | Isolates |
|--------|------------|----------|
| 1      | Sparse (9 -15) | Mp 2, Mp 3 |
| 2      | Medium (15.1-21.0) | Mp 1, Mp 5 |
| 3      | Abundant (more than 21.0) | Mp 4, Mp 6, Mp 7, Mp 8, Mp 9, Mp 10 |

### Table 2.3: Grouping of isolates of *Macrophomina phaseolina* on the basis of shape of the sclerotia

| Sl. No | Characters | Isolates |
|--------|------------|----------|
| 1      | Round | Mp 3 |
| 2      | Ovoid | Mp 1, Mp 2, Mp 4, Mp 5, Mp 6, Mp 9, |
| 3      | Irregular | Mp 7, Mp 8, Mp 9 |

### Table 3: Variability in cultural characteristics of *Macrophomina phaseolina* isolates

| Sl. No | Isolate Code | Growth Rate (cm) | Pigmentation | Colony texture |
|--------|--------------|------------------|--------------|----------------|
| 1      | Mp 1         | 7.50             | Black        | PS             |
| 2      | Mp 2         | 7.20             | Light Grey   | PS             |
| 3      | Mp 3         | 8.70             | Light Grey   | F              |
| 4      | Mp 4         | 8.57             | Light Grey   | S              |
| 5      | Mp 5         | 8.00             | Grey         | PS             |
| 6      | Mp 6         | 7.90             | Light Grey   | PS             |
| 7      | Mp 7         | 6.80             | Black        | PS             |
| 8      | Mp 8         | 7.60             | Grey         | PS             |
| 9      | Mp 9         | 7.80             | Grey         | PS             |
| 10     | Mp 10        | 8.40             | Black        | PS             |

| C.D.  | 0.34 |
| SE(m) | 0.11 |
| C.V.  | 2.55 |

F: Fluffy PS: Partially submerged S: Submerged

### Table 3.1: Grouping of isolates of *R. bataticola* on the basis of mycelial growth

| Sl. No | Characters | Isolates |
|--------|------------|----------|
| 1      | Submerged | Mp 4 |
| 2      | Partially Submerged | Mp 1, Mp 2, Mp 5, Mp 6, Mp 7, Mp 8, Mp 9, |
| 3      | Fluffy | Mp 3 |

### Table 4: Pathogenic Variability of *Macrophomina phaseolina* isolates

| Sl. No | Isolate Code | Percent Disease Incidence (%) | Virulence category |
|--------|--------------|-------------------------------|--------------------|
| 1      | Mp 1         | 66.66 (54.71) *               | Highly Virulent    |
| 2      | Mp 2         | 63.64 (52.90)                 | Highly Virulent    |
| 3      | Mp 3         | 46.00 (42.68)                 | Virulent           |
| 4      | Mp 4         | 70.47 (57.07)                 | Highly Virulent    |
| 5      | Mp 5         | 59.82 (50.64)                 | Highly Virulent    |
| 6      | Mp 6         | 73.33 (58.89)                 | Highly Virulent    |
| 7      | Mp 7         | 57.31 (49.18)                 | Highly Virulent    |
| 8      | Mp 8         | 53.33 (46.89)                 | Highly Virulent    |
| 9      | Mp 9         | 60.00 (50.75)                 | Highly Virulent    |
| 10     | Mp 10        | 44.35 (41.73)                 | Virulent           |

| C.D.  | 2.55 |
| SE(m) | 0.86 |
| C.V.  | 2.95 |

* Values in the parentheses are angular transformed and are the means of three replications
Fig 4: Variability in pathogenicity of ten *Macrophomina phaseolina* isolate

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