



## Research Article

# CULTURAL AND MORPHOLOGICAL VARIABILITY OF *MACROPHOMINA PHASEOLINA* CAUSED BY SOYBEAN

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**Abstract-** Charcoal rot caused by the fungus have emerged as serious concern for cultivation of soybean under climate change scenario worldwide. In the present studies, 16 isolates of *Macrophomina phaseolina* from different regions of Madhya Pradesh were analyzed for Cultural and morphological variability. The parameters can be observed from results that Colony characteristics, length, width and number of sclerotia of sixteen isolates of *Macrophomina phaseolina* grown on P.D. A.

**Keywords-** Soybean *Macrophomina phaseolina* isolates, Cultural and morphological variability

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## Introduction

Soybean, *Glycine max* (L.) Merrill, is susceptible to a large number of disease agents such as seedling and root pathogens that cause serious damages to this crop. One of these soil borne pathogens is *Macrophomina phaseolina* (Tassi) Goid, the causal agent of charcoal root rot. This polyphagous pathogen attacks and infects more than 500 plant species in 100 families of monocots and dicots [1]. In India 70 percent loss cause by charcoal rot has been reported. The disease is distributed in Madhya Pradesh, Maharashtra, Rajasthan and Delhi. In M.P. the crop sequence gram – soybean and dry spell during pod filling have played major role in enhancing the incidence of charcoal rot of soybean caused by *Macrophomina phaseolina*. There are reports in other parts of the world that populations of *M. phaseolina* showed significant variations morphologically [2], physiologically [3] pathogenically [4] and genetically [5]. These variations aid the pathogen to adapt and survive in diverse environments. Therefore the present investigation is under taken with the following.

## Material and Methods

Experiment and related studies conducted in the Department of Plant Pathology, JNKVV and Jabalpur.

## Variability among isolates of *Macrophomina phaseolina*

### Cultural variability

The cultural characteristics of *Macrophomina phaseolina* were recorded by growing isolates on PDA. (15-17) ml of PDA was poured in each of previously sterilized Petri plates. Five mm discs were cut by sterilized cork borer from the margin of seven days old colony. One disc was placed in the centre of each plate and incubated at 25± 1°C for seven days. Three replications were maintained for each isolate.

### Morphological variability

The slides of various isolates were prepared in lacto phenol from 10days old culture for morphological studies Ten hundred observations for size of sclerotia and size of hyphal width and number of sclerotia per microscopic field belonging to each isolates were taken low power (10x x 10x) microscopic field.

These isolates were categorized in various groups according to size of hyphal width, size of sclerotia, number of sclerotia, colour of sclerotia and shape of sclerotia.

Disease samples of soybean collected from various districts of Madhya Pradesh state

District	Locality of collocation of isolates	Designation of isolates of <i>Macrophomina phaseolina</i>
Jabalpur	Adhartal	I <sub>1</sub>
Vidisha (Ganjbasoda)	K.V.K Farmer field	I <sub>2</sub> I <sub>3</sub>
Narsingpur	K.V.K	I <sub>4</sub>
Sagar	K.V.K Farmer field	I <sub>5</sub> I <sub>6</sub>
Narsingpur	Farmer field	I <sub>7</sub>
Jabalpur	Farmer field	I <sub>8</sub>
Narsingpur (Gadarwara)	Farmer field	I <sub>9</sub>
Jabalpur	Khamariya Krishi Nagar Farm Krishi Nagar Farm	I <sub>10</sub> I <sub>11</sub> I <sub>12</sub>
Indore	K.V.K.	I <sub>13</sub>
Sagar (Garhakota)	Farmer field	I <sub>14</sub>
Rewa	K.V.K	I <sub>15</sub>
Tikamgarh	K.V.K	I <sub>16</sub>

## Results and Discussion

### Mean radial growth of *Macrophomina phaseolina* 16 isolates on P.D.A. medium incubate at 25 ± 1°C

Data on Radial growth recorded [Table-1] Every alternate day's showed that after 48 hrs, of inoculation it ranged from 23.2 to 44.2 mm. Minimum growth of 23.2 and 28.2 was recorded in isolate I<sub>13</sub> (Indore, KVK) and I<sub>16</sub> (Tikamgarh, KVK) respectively. Significantly highest growth of 44.2 mm and 42.2 mm was noted in I<sub>10</sub> (Jabalpur, Khamariya) and I<sub>1</sub> (Jabalpur, Adhartal) isolates. Whereas data recorded after 72 and 120 hrs, showed narrow variation 58.4 to 74.0 mm and 76.8 to 84.6 mm respectively. Two isolates I<sub>13</sub> (Indore, KVK) and I<sub>16</sub> (Tikamgarh, KVK) fell into the category of slow growing and I<sub>10</sub> (Jabalpur, Khamariya) and I<sub>1</sub> (Jabalpur, Adhartal) as fast growing isolates.

Table-1 Mean radial growth of *Macrophomina phaseolina* 16 isolates on P.D.A. medium incubate at  $25 \pm 1^\circ\text{C}$ .

Isolate No.	48 hrs.	72 hrs.	120 hrs.	Colony character
I <sub>1</sub> Jabalpur (Adhartal)	42.2	74.2	78.2	Black colour and fluffy growth in middle ring.
I <sub>2</sub> Ganjbasoda (KVK)	32.4	64.4	76.2	Grey colour slightly fluffy
I <sub>3</sub> Ganjbasoda (Farmer field)	34.6	74.8	78.2	Dark black and dense growth
I <sub>4</sub> Narsinghpur (KVK)	37.0	72.4	79.4	Greyish black and dense growth
I <sub>5</sub> Sagar (KVK)	37.2	70.6	82.4	Greyish black slightly fluffy
I <sub>6</sub> Sagar (Farmer field)	31.0	72.4	79.6	Greyish black fluffy growth
I <sub>7</sub> Narsinghpur (Farmer field)	33.6	59.6	79.0	Dark black slightly fluffy
I <sub>8</sub> Jabalpur (Farmer field)	38.8	65.6	76.8	Dark grey and fluffy growth
I <sub>9</sub> Gadarwara (Farmer field)	31.8	62.4	79.2	Whitish grey and feathery
I <sub>10</sub> Jabalpur (Khamariya)	44.2	71.8	83.2	Dark grey and feathery
I <sub>11</sub> Jabalpur (Krishi nagar farm)	34.2	58.4	84.4	Black and feathery
I <sub>12</sub> Jabalpur (Krishi Nagar Farm)	38.6	66.6	83.8	Black colour and feathery
I <sub>13</sub> Indore(KVK)	23.2	67.0	78.2	Black and feathery growth
I <sub>14</sub> Garhakota (Farmer field)	36.6	63.4	78.8	Black feathery
I <sub>15</sub> Rewa (KVK)	31.2	71.4	84.6	Whitish grey and feathery
I <sub>16</sub> Tikamgarh (KVK)	28.2	69.8	83.0	Greyish fluffy
SEm±	0.521	0.512	0.609	
CD%	1.506	1.482	1.761	

Table-2 Different soybean isolates of *Macrophomina phaseolina* from Madhya Pradesh Categorization of on the basis of radial growth of 48 hrs.

S	Category	Numbers	Isolates
1	Slow growing (56.0 × 56.0 to 70.0 to 70.0 mm)	2	I <sub>13</sub> , I <sub>16</sub>
2	Medium growing (70.0 × 70.0 to 80.0 × 70.0 mm)	12	I <sub>2</sub> , I <sub>3</sub> , I <sub>4</sub> , I <sub>5</sub> , I <sub>6</sub> , I <sub>7</sub> , I <sub>8</sub> , I <sub>9</sub> , I <sub>11</sub> , I <sub>12</sub> , I <sub>14</sub> , I <sub>15</sub>
3	Fast growing (91.0 × 70.0 to 95.0 × 70.0 mm)	2	I <sub>1</sub> , I <sub>10</sub>

Table-3 Categorization of *Macrophomina phaseolina* on the basis of colony character.

S	Category	Numbers	Isolates
1	Dark black and feathery	4	I <sub>3</sub> , I <sub>7</sub> , I <sub>8</sub> , I <sub>10</sub>
2	Greyish black and feathery	10	I <sub>1</sub> , I <sub>2</sub> , I <sub>4</sub> , I <sub>5</sub> , I <sub>6</sub> , I <sub>11</sub> , I <sub>12</sub> , I <sub>13</sub> , I <sub>14</sub> , I <sub>16</sub>
3	Whitish grey and feathery	2	I <sub>9</sub> , I <sub>15</sub>

Table-4 Mean length, width and number of sclerotia of sixteen isolates of *Macrophomina phaseolina* grown on P.D. A. incubated at  $25^\circ\text{C}$  for 10 days.

Isolates Number		Size of Sclerotia (µm)					
Number of sclerotia (mm) /microscopic field) (10X10)		Min. Length	Min. Width	Max. Length	Max. Width	Avg. Length	Avg. Width
I <sub>1</sub>	15.0	47.0	47.0	94.0	94.0	68.6	69.5
I <sub>2</sub>	14.0	56.4	56.4	84.6	84.6	70.5	67.6
I <sub>3</sub>	16.4	56.4	56.4	84.6	84.6	75.2	75.2
I <sub>4</sub>	20.2	47.0	56.4	84.6	84.6	63.9	67.6
I <sub>5</sub>	15.2	56.4	56.4	112.8	112.8	78.0	77.0
I <sub>6</sub>	16.0	56.4	56.4	84.6	84.6	71.4	71.4
I <sub>7</sub>	12.6	47.0	56.4	103.4	112.8	72.3	74.2
I <sub>8</sub>	19.2	47.0	47.0	103.4	94.0	62.9	61.1
I <sub>9</sub>	16.2	56.4	56.4	122.2	122.2	85.5	84.6
I <sub>10</sub>	20.5	56.4	56.4	84.6	75.2	67.6	64.8
I <sub>11</sub>	12.8	47.0	47.0	84.6	84.6	62.9	63.9
I <sub>12</sub>	22.4	47.0	47.0	141.0	131.6	82.7	80.8
I <sub>13</sub>	23.0	65.8	65.8	84.6	84.6	71.4	74.2
I <sub>14</sub>	12.0	56.4	47.0	94.0	94.0	72.3	72.3
I <sub>15</sub>	11.9	47.0	47.0	103.4	103.4	77.0	78.0
I <sub>16</sub>	14.7	47.0	47.0	65.8	65.8	56.4	56.4

Table-5 Categorization of *Macrophomina phaseolina* on the basis of number of Sclerotia per microscopic field.

Serial No.	Category	Numbers	Isolates
1	Low(10-15)	7	I <sub>1</sub> , I <sub>2</sub> , I <sub>7</sub> , I <sub>11</sub> , I <sub>14</sub> , I <sub>15</sub> , I <sub>16</sub>
2	Medium(16-20)	5	I <sub>3</sub> , I <sub>5</sub> , I <sub>6</sub> , I <sub>8</sub> , I <sub>9</sub>
3	Abundant>20	4	I <sub>4</sub> , I <sub>10</sub> , I <sub>12</sub> , I <sub>13</sub>

Table-6 Categorization of *Macrophomina phaseolina* on the basis of size of Sclerotia.

Serial No.	Category	Numbers	Isolates
1	Small (50-60 μm)	1	I <sub>16</sub>
2	Medium (61.070.0 μm)	13	I <sub>1</sub> , I <sub>2</sub> , I <sub>3</sub> , I <sub>4</sub> , I <sub>5</sub> , I <sub>6</sub> , I <sub>7</sub> , I <sub>8</sub> , I <sub>9</sub> , I <sub>10</sub> , I <sub>11</sub> , I <sub>12</sub> , I <sub>13</sub> , I <sub>14</sub>
3	Large (>71.0 μm)	2	I <sub>8</sub> , I <sub>15</sub>

Most of the isolates had grayish black and feathery growth whereas I<sub>9</sub> (Gadarwara, Farmer field) and I<sub>15</sub> (Rewa, KVK) showed whitish grey and feathery colony. Isolates I<sub>3</sub> (Ganjbasoda, KVK), I<sub>7</sub> (Narsinghpur, Farmer field), I<sub>8</sub> (Jabalpur Farmer field) and I<sub>10</sub> (Jabalpur, khamariya) had dark black and feathery growth.

On the basis of radial growth, the isolates were categorized as Fast (91.0 × 70.0 to 95.0 × 70.0 mm) medium (70.0 × 70.0 to 80.0 × 70.0 mm) and small (56.0 × 56.0 to 70.0 to 70.0 mm) growing. Out of 16 isolates, 2 isolates were fell into the category of slow, 12 in medium and 2 in fast growing [Table-2].

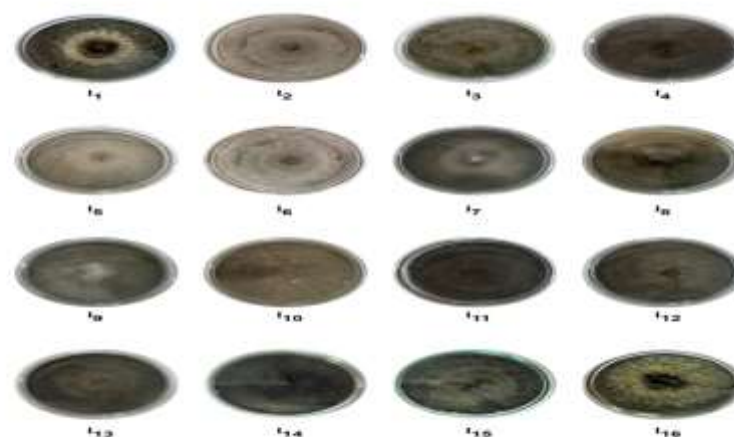
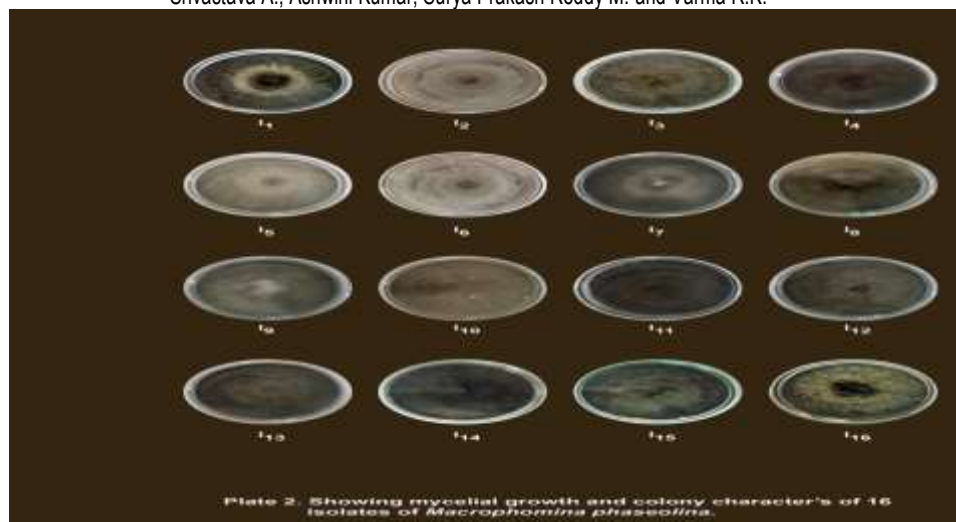


Plate 2: Showing mycelial growth and colony character's of 16 isolates of *Macrophomina phaseolina*.

#### Categorization of *Macrophomina phaseolina* on the basis of colony character.

On the basis of colony character, the isolates were categorized as Dark black and feathery, Greyish black and feathery, Whitish grey and feathery. Out of 16 isolates, 4 isolates were fell into the category of dark black and feathery, 10 in greyish black and feathery and 2 in whitish grey and feathery [Table-3]. In the present investigation sixteen isolates were grown on PDA. The results indicated the variation in the rate of growth and colony character. On the basis of rate of growth sixteen isolates were classified into three categories (slow, medium and fast growing) and on the basis of colony character it was divided into the three categories i.e. (dark black and feathery, greyish black and feathery, whitish grey and feathery). Similar finding has been recorded [6-8].

#### Mean length, width and number of sclerotia of sixteen isolates of *Macrophomina phaseolina* grown on P.D. A. incubated at $25 \pm 1^\circ\text{C}$ for 10 days.

The data presented in showed variation in number of sclerotia per microscopic field ( $10\times \times 10\times$ ) from 11.9 to 23.1 per microscopic field. Lowest number of 11.9 and 12.0 was recorded in isolates I15 (Rewa, KVK) and I14 (Garhakota, Farmer Field) whereas highest number of 23.1 and 22.4 is isolates I13 (Indore KVK) and I11 (Jabalpur, Krishi nagar farm). Data on sclerotial size indicated that minimum length ranged from 47.0 to 75.2  $\mu\text{m}$  and minimum width 47.0 to 65.8  $\mu\text{m}$  among 16 isolates. Maximum length and maximum width varied from 65.8 to 141.0  $\mu\text{m}$  and 65.8 to 131.6  $\mu\text{m}$ . Variation in length was more as compared to variation in width. Among isolates had small average size of  $56.4 \times 56.4$  was recorded in isolate I16 (Tikamgarh, KVK) and largest size in isolate I15 (Rewa, KVK) and I8

(Jabalpur, Farmer field)  $92.1 \times 78.0$  and  $75.2 \times 62.9$   $\mu\text{m}$  respectively. All other 14 isolates had medium range [Table-4].

#### Categorization of *Macrophomina phaseolina* on the basis of number of Sclerotia per microscopic field.

Sclerotial population in different isolates was calculated by observing the 5 mm disc of ten days old culture of *M. Phaseolina* under microscope. The isolates were classified abundant ( $>20$ ), medium (15-20) and low (10-15) sclerotial population. Out of 16 isolates, 7 isolates were fell into the category of low, 5 in medium and 4 in abundant [Table-5].

#### Categorization of *Macrophomina phaseolina* on the basis of size of Sclerotia.

Slides from 10 days old pure cultures of *Macrophomina phaseolina* isolates were prepared and examined under a microscope with ocular micrometer. The isolates were classified as large ( $>71.0\mu\text{m}$ ), medium, (61.0-70.0 $\mu\text{m}$ ) and small (50-60  $\mu\text{m}$ ) sized sclerotia. Categorization made under size of sclerotia only one isolate I16 (Tikamgarh KVK) and two isolates I18 and I15 were categorized as small and large sclerotia respectively. Other 13 isolates had medium sized sclerotia [Table-6]. According to number of sclerotia per microscopic field and size of sclerotia. The sixteen isolates were categorized into three groups for no. of sclerotia (low, medium and abundant) and for size of sclerotia (small, medium and large). Similar categorization and variability no. of sclerotia and size also be reporting by various author in various crop [9]. In soybean from Madhya Pradesh and Iqbal and [3] in mungbean from Pakistan. Therefore, the present findings are in accordance with the finding of three workers.

**Application of research:** Study of variability among isolates of *Macrophomina phaseolina*

**Research Category:** Plant Pathology

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**Study area / Sample Collection:** Department of Plant Pathology, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Adhartal, Jabalpur, 482004, Madhya Pradesh

**Cultivar / Variety / Breed name:** Soybean, *Glycine max* (L.) Merrill

**Conflict of Interest:** None declared

**Ethical approval:** This article does not contain any studies with human participants or animals performed by any of the authors.

Ethical Committee Approval Number: Nil

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