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Cultural and morphological variability among the isolates of *Macrophomina phaseolina* (Tassi) Goid. Causing stem and root rot of sesame (*Sesamum indicum* L.)

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Abstract
Cultural and morphological variability of different isolates of *M. phaseolina* collected from four locations of Gujarat *viz*., Anand, Junagadh, S. K. Nagar, Godhra and one from Raipur district of Chhattisgarh was studied on three different solid media *viz*., Potato dextrose agar, Oat meal agar and Host leaf extract agar revealed the considerable variation among the isolates of *M. phaseolina* indicated the existence of variability in the pathogen. Moreover, PDA was found as an excellent media to support the growth and sclerotial formation of isolates of *M. phaseolina*. Out of three media tested, Potato dextrose agar proved to be the best medium in respect of radial growth of pathogen for all the isolates. In case of isolates, Junagadh (Mp 2) and S. K. Nagar (Mp 3) isolates exhibited highest mean radial growth (90.00 mm). Among all the isolates of *M. phaseolina*, distinct differences in terms of type of margin, topography of colony, color of colony, size of sclerotia and shape of sclerotia were observed. The average sclerotial size of all isolates varied from 62.93-129.36 µm x 46.93-111.28 µm.

Keywords: Sesame, *Macrophomina phaseolina*, stem and root rot, cultural and morphological variability, potato dextrose agar, oat meal agar, host leaf extract agar

Introduction
Sesame (*Sesamum indicum* L.) is an ancient and traditional oilseed crop of India, cultivated in about. 74 million hectare area and producing 0.82 million tons. The main reason for the low productivity of sesame is due to the attack of various diseases. Among the fungal diseases, stem and root rot also called charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. is widely distributed and highly destructive right from the establishment phase of crop (Dinakaran and Mohammed, 2001) [5], causing up to 50 per cent or more disease incidence in field resulting in heavy yield losses (Chattopadhyay *et al*., 2002) [2]. Yield losses have been estimated up to 57 per cent when there is about 40 per cent infection (Maiti *et al*., 1988) [8] and about 5-100% yield loss as estimated by Vyas (1981). Further loss in yield at the rate of 1.8 kg/ha due to 1 per cent increase in the incidence has been reported (Murugesan *et al*., 1978) [9]. *Macrophomina phaseolina* (Tassi) Goid is one of the most destructive necrotrophic fungal pathogens that infect more than 500 plant species across 75 families. Under moisture stress condition, the fungus causes many diseases like seedling blight, collar rot, stem rot, charcoal rot and dry root rot. The most common symptoms of the disease are the sudden wilting of plants throughout the crop growth mainly after the flowering phase. The pathogen attacks mostly at the basal region of the plant (Kumar *et al*., 2011) [7].
Not much work has been done on the variability among the isolates of *M. phaseolina* on sesame and needs special attention, since breeding for disease resistance is based on the knowledge of existence of variation in the pathogen population. In the present investigation, information on cultural and morphological variation of *M. phaseolina* is reported.

Materials and Methods
The typical stem and root rot infected samples (five isolates) were collected from major sesame growing areas of Gujarat and one isolate from Raipur district of Chhattisgarh and isolations were made to study the cultural and morphological variations among the different isolates.

Determination of Cultural Variability

Cultural variability among the isolates of *M. phaseolina* was studied based on following characters.

Growth Character of Solid Media : The growth characters of different isolates of *M. phaseolina* was studied on three different solid media *viz*., Potato dextrose agar, Oat meal agar and Host leaf extract agar.

The compositions of above culture medium areas under**:**

(1) Potato Dextrose Agar (PDA)

Peeled potatoes	:	200g
Agar agar	:	20g
Dextrose ($C_6H_{12}O_6$)	:	20g
Distilled water	:	1000ml

(2) Oat Meal Agar (OMA)

Oat meal	:	100g
Agaragar	:	20g
Trace salt solution	:	1ml
Distilled water	:	1000ml

(3) Host Leaf Extract Agar (HLEA)

Host leaf bits	:	200g
Agar agar	:	20g
Dextrose ($C_6H_{12}O_6$)	:	20g
Distilled water	:	1000ml

Twenty milliliter of each of the medium was poured into each of sterilized Petri plates. Inoculation was made by transferring the 5 mm disc of mycelia mat, taken from the periphery of 10 days old culture of different isolates. Each treatment was repeated thrice. The plates were incubated at 28 ± 1^0C for 10 days. Observation on fungal radial growth (mm) was recorded when the maximum growth was attained in any one of the media tested. Other cultural characters *viz.*, type of margin, topography of colony, color of colony and sclerotial production were also recorded.

Sclerotial Formation: The sclerotial formation of the pathogen on different media were assessed by microscopic observations. A loopful of one-week-old culture was transferred to a clean glass slide and mixed well with lactophenol and place cover slip on it. The sclerotial formation (Table 1.) was recorded in five different microscopic fields.

Table 1: Categories of sclerotial formation

Sr. No.	Rate of sclerotial formation	No. of sclerotia / microscopic field (10x)	Category
1	No Sclerotial formation	0	-
2	Poor	1-10	+
3	Moderate	11-20	++
4	Good	21-30	+++
5	Excellent	>30	++++

Determination of Morphological Variability

Morphological variability among the isolates of *M. phaseolina* was studied based on following characters.

The morphological characters of different isolates of *M. phaseolina* including shape of sclerotia, size of sclerotia (µm) and number of sclerotia/ microscopic field of 10x were measured. The photomicrographs were also taken by using camera attachment binocular microscope to show the typical morphology of sclerotia of the isolates.

For measuring sclerotial size, slides from seven days old pure cultures of *M. phaseolina* isolates were prepared and examined under high power objective (45x). The sizes of 10 randomly selected sclerotia were measured using 'SImage 2013 Beta' software and their means were calculated (Iqbal and Mukhtar, 2014).

Results and Discussion

The result presented in Table 2 revealed that there was a considerable variations among the colony characteristics of the different isolates on three different solid media.

(i) Potato dextrose agar (PDA)

The maximum radial growth (90 mm) was found in Junagadh (Mp2) isolate and S. K. Nagar (Mp3) isolate among all five isolates.

Colony margin of all isolates were different *viz*., Anand (Mp 1) isolate showed irregular and scattered margin, Junagadh (Mp 2) and Godhra (Mp 4) isolate showed luxuriant uniform, while S. K. Nagar (Mp 3) isolate showed dense uniform and Raipur (Mp 5) isolate showed dense irregular margin of colony.

All the isolates showed different topography of colony. Anand (Mp 1) isolate and Godhra (Mp 4) isolate showed fluffy growth, Junagdh (Mp 2) isolate showed dense uniform and circular, while S. K. Nagar (Mp3) and Raipur (Mp4) isolates showed flat and dense growth.

All five isolates were showed black, grayish black to dark black color of colony (Table 2).

(ii) Oat meal agar (OMA)

The maximum radial growth (90 mm) was found in Junagadh (Mp2) isolate and S. K. Nagar (Mp3) isolate among all five isolates.

Colony margin of all the isolates were different *viz*., Anand (Mp 1) isolate showed irregular and scattered, Junagadh (Mp 2) and Godhra (Mp 4) isolate showed luxuriant uniform, S. K. Nagar (Mp 3) isolate showed luxuriant and Raipur (Mp 5) isolate showed dense irregular margin of colony.

Anand (Mp 1), Godhra (Mp 4) and Raipur (Mp 5) isolates showed dense and flat topography, while Junagadh (Mp 2) and S. K. Nagar (Mp 3) isolates showed fluffy growth.

Colony color of all the isolates were different *viz*., Anand (Mp 1) isolate showed dark black, Junagadh (Mp 2) isolate showed gray, S. K. Nagar (Mp 3) isolate showed light gray, while Godhra (Mp 4) and Raipur (Mp 5) isolates showed purple color of colony (Table 2).

(iii) Host leaf extract agar

The maximum radial growth (90 mm) was found inJunagadh (Mp2) isolate and S. K. Nagar (Mp3) isolate among all five isolates.

Colony margin of Anand (Mp 1) isolate was irregular and scattered, Junagadh (Mp 2) and S. K. Nagar (Mp 3) isolates showed luxuriant uniform, while Godhra (Mp 4) and Raipur (Mp 5) isolates showed luxuriant irregular margin of colony.

Table 2: Cultural variability among the different isolates of *M. phaseolina* on different solid media

Sr. No.	Isolates	Culture Media	Radial growth (mm)*	Type of margin	Topography of colony	Color of colony
1	Mp 1 (Anand)	Potato dextrose agar	75.35	Irregular and scattered	Fluffy	Grayish black
		Oat meal agar	73.27	Irregular and scattered	Dense and flat	Dark black
		Host leaf extract agar	58.87	Irregular and scattered	Fluffy	Black
2	Mp 2 (Junagadh)	Potato dextrose agar	90.00	Luxuriant uniform	Dense uniform and circular	Black
		Oat meal agar	90.00	Luxuriant uniform	Fluffy	Gray
		Host leaf extract agar	90.00	Luxuriant uniform	Fluffy	Grayish black
3	Mp 3 (S. K. Nagar)	Potato dextrose agar	90.00	Dense uniform	Flat and dense	Grayish black
		Oat meal agar	90.00	Luxuriant	Fluffy	Light gray
		Host leaf extract agar	90.00	Luxuriant uniform	Fluffy	Grayish black
4	Mp 4 (Godhra)	Potato dextrose agar	83.73	Luxuriant uniform	Fluffy	Black
		Oat meal agar	65.28	Luxuriant uniform	Dense and flat	Purple
		Host leaf extract agar	82.20	Luxuriant irregular	Fluffy	Black
5	Mp 5 (Raipur)	Potato dextrose agar	85.63	dense irregular	Dense and flat	Dark black
		Oat meal agar	82.67	dense irregular	Dense and flat	Purple
		Host leaf extract agar	79.08	Luxuriant irregular	Fluffy scattered	Black

*Mean of three repetitions

Table 3: Morphological variability among the different isolates of *M. phaseolina* on different solid media

Sr. No.	Isolates	Culture Media	Size of sclerotia (µm)		Shape of sclerotia	Sclerotial production
			Length (µm)	Width (µm)		
1	Mp 1 (Anand)	Potato dextrose agar	122.20	111.28	Irregular	++++
		Oat meal agar	70.02	56.04	Irregular	++
		Host leaf extract agar	126.21	92.67	Oval	+
2	Mp 2 (Junagadh)	Potato dextrose agar	89.26	46.93	Circular to oval	++++
		Oat meal agar	64.10	54.27	Circular	+++
		Host leaf extract agar	129.36	86.92	Circular to oval	+
3	Mp 3 (S. K. Nagar)	Potato dextrose agar	94.90	64.04	Circular to oval	++++
		Oat meal agar	96.91	75.02	Circular	++
		Host leaf extract agar	82.23	71.51	Circular	+
4	Mp 4 (Godhra)	Potato dextrose agar	118.28	81.45	Circular to kidney shaped	++++
		Oat meal agar	85.05	72.34	Circular	+
		Host leaf extract agar	95.55	71.23	Circular, oval and rectangular	++
5	Mp 5 (Raipur)	Potato dextrose agar	62.93	53.09	Oval	++++
		Oat meal agar	115.54	98.37	Irregular	++
		Host leaf extract agar	117.61	88.55	Circular to irregular	++

Note: + = Poor, ++ = Moderate, +++ = Good, ++++ = Excellent

All five isolates showed fluffy to scattered fluffy topography and showed black to grayish black color of colony (Table 2).

These observations are in conformity with the finding of earlier workers (Devi and Singh, 1998; Shekhar *et al*., 2006; Deepthi *et al*. 2014a) [4, 11, 3]. Diversity in cultural characters such as colony color, its margins and topography were noticed among the isolates of *M. phaseolina*.

Several workers (Iqbal and Mukhtar, 2014; Ashraf *et al*. 2015; Sukanya *et al*. 2016) [6, 1] also observed diversity in cultural characteristics such as growth rate, type of growth, colony color and sclerotial formation among the different isolates of *M. phaseolina.*

Determination of Morphological Variability

The result presented in Table 3 revealed that there was a considerable variations among the morphological characteristics *viz.,* sclerotial production, size of sclerotia and shape of sclerotia of the isolates of *M. phaseolina* on three different solid media.

Based on size of sclerotia, different isolates and different media were grouped into different groups. Different sizes of sclerotia *viz.,* 62.93-129.36µm (length) x46.93-111.28 µm (width) were recorded. Based on shape of sclerotia, isolates and media were grouped into different groups. Different shape of sclerotia *viz.,* irregular, oval, circular to oval, circular, circular to kidney shaped, rectangular and circular to irregular were recorded.

All the isolates were showed excellent sclerotial production on potato dextrose agar media. Good to moderate sclerotial production on oat meal agar, while moderate to poor sclerotial formation on host leaf extract agar.

The result revealed that potato dextrose agar was found most suitable medium for growth and sclerotial production of the pathogen.

The sclerotial morphology of isolates of *M. phaseolina* are in accordance with those described by Shekhar *et al.* (2006) [11], Prasad *et al.* (2011) [10], who reported differences among the isolates of *M. phaseolina* in terms of size and shape of sclerotia and sclerotial production.

The more or less similar results were also described by Ashraf *et al.* (2015) [1] and Sukanya *et al.* (2016). Thus, the present findings tallied with the studies carried out by earlier workers.

Morphological variations *i.e.* sclerotial production, size and shape of sclerotia in the isolates of *M. phaseolina* could be due to nutrition rather than a characteristics pathological variation. However, in the present studies glaring differences in the sclerotial size and shape were noticed among the isolates even when same medium was used for the growth of the isolates. It can be assumed that variation in the isolates may be inherent, since isolates were collected from diverse agroclimatic zones of Gujarat and Chhattisgarh. Hence, these variations in the sclerotial size and shape indicated the existence of variability in this pathogen.

Even though only one species was recognized within the genus *Macrophomina*, great variability in cultural and morphology were recorded.

The results suggested the prevalence of high degree of variability in the cultural and morphological variables in *M. phaseolina* isolates.

The determination of variability among the *M. phaseolina* isolates is fundamental to guide the development of appropriate strategies for disease management according to different agroecological zones. The present studies provide information on the variability of *M. phaseolina* in major sesame growing areas. These results will be useful in developing integrated strategies for the management of stem and root rot and breeding programme for oilseed crops.

Conclusion

Out of three media tested, potato dextrose agar was found an excellent media for growth and sclerotial formation of isolates of *M. phaseolina*. In case of isolates, Junagadh (Mp2) and S. K. Nagar isolate (Mp3) exhibited highest mean radial growth (90.00 mm) on all the media. Oat meal agar (73.27 and 82.67 mm) proved to be better to host leaf extract agar (58.87 and 79.08 mm) for Anand isolate (Mp 1) and Raipur isolate (Mp 5), respectively, while for the Godhra isolate (Mp 4), host leaf extract agar (82.20 mm) showed better radial growth than oat meal agar (65.28 mm). Among five isolates of *M. phaseolina*, district differences in terms of type of margin, topography of colony, size and shape of sclerotia were observed. The average sclerotial size was varied from 62.93-129.36µm x46.93-111.28µm.

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