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SURVEY, CULTURAL CHARACTERS AND PATHOGENICITY OF *MACROPHOMINA PHASEOLINA* CAUSING SESAME ROOT ROT DISEASE

Thirunarayanan P., Sanjaygandhi S*., Vengadeshkumar L., Rajamohan K. and R. Udhayakumar

Department of Plant Pathology, Faculty of Agriculture, Annamalai University, AnnamalaiNagar, Chidambaram-608002 (Tamil Nadu) India.

Abstract

Sesame root rot is caused by *M. phaseolina* also one of the major factor of sesame production constrain. Hence, the present study was conducted with an objective to assess the prevalence and incidence of root rot disease in different locations of cuddalore district, Tamil Nadu, India during 2017 and assess the cultural characters and pathogenic variability among the isolates of *M. phaseolina*. The survey clearly revealed that the disease incidence ranged from 13.60 to 34.65 per cent showing the endemic nature of the disease. Among the isolates MP_4 was found as the most virulent which recorded maximum percent disease incidence (46.65%) on sesame variety PKM 3 with artificial inoculation. Also, the same isolate was found to be as best in radial growth (90.00), mycelial character (Black profusely aerial growth) and sclerotial characters *viz*., size (104.18 µ) and number (188.46) of sclerotia.

Key words: Root rot; survey; *Macrophomina phaseolina*; characterization.

Introduction

Sesame (*Sesamum indicum* L.) is variously named as gingelly, till or tila. The genus Sesamum belongs to the family Pedaliaceae and order Tubiflora which comprises 16 genera and 60 species (Weiss, 1983). It is considered as most important oilseed crop grown in tropical and subtropical areas of the world (Hamza and Salam, 2015). It is also a rich source of vitamins (pantothenic acid and vitamin E) and phosphorus (570mg/100g) (Balasubramaniyan and Palaniappan, 2001). In world, Sesame is grown in area of 11.25 million hectares with production of 6235.53 thousand tones and productivity of 576.3 kg/ha (FAO, 2014) Globally, India is the largest producer, consumer and exporter of sesame as per the Solvent Extractions Association of India (SEAI), the area under sesame crop is 19.81 lakh hectares with production of 8.87 lakh tones during 2015-2016. Among the various diseases, root rot caused by *Macrophomina phaseolina* (Tassi) Goid. is the most serious one affecting the crop at later stages of growth (Buldo and Rane, 1978), with reports upto 50 percent incidence resulting heavy yield losses (Chattopadhyay and Kalpanasastry, 1998). *M. phaseolina* has been recently reported as an emerging phytopathogen (Kaur *et al*., 2012). It has been reported that the disease causes yield loss up to the extent of 30-40 percent in Tamil Nadu (Balabaskar, 2006; Ndiaye, 2007; Sarr *et al*., 2014; Savaliya *et al.*, 2015). Thus, root rot disease is very destructive to sesame crop the present study was conducted with an objective to assess the prevalence and incidence of root rot of sesame in different regions of Cuddalore district, Tamil Nadu, India and assess the cultural characters and pathogenic variability among the isolates of *M. phaseolina*.

***Author for correspondence :** E-mail: premasivajothi@gmail.com

Materials and Methods

Survey for the incidence of sesame root rot

A field survey was conducted to assess the extent of root rot occurrence of sesame in Cuddalore district, Tamil Nadu, India. Seven locations representing both rainfed and irrigated situations were selected for the study. The per cent disease index was worked out using the 0 to 9 scale according to "Phytopathometry" by Mayee and Datar, (1986) as mentioned below.

Disease scale,

0 - no symptoms on any plant

1 - 1% or less plants killed

3 - 1-10% plants killed

5 - 11-20% plants killed

7 - 21-50% plants killed

9 - 51% or more plants killed

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of disease incidence}}{\text{Total no. of plants observed}} \times \frac{100}{\text{Maximum grade used}}$$

The other information *viz*., soil type and the crop variety were also recorded in the respective surveyed fields.

Isolation of the pathogen

M. phaseolina was isolated from the root rot infested sesame plants showing the typical root rot symptoms by tissue segment method (Rangaswami, 1972) on potato dextrose agar (PDA) medium. The axenic cultures of the pathogen were obtained by single hyphal tip method (Rangaswami, 1972) and these were maintained on PDA slants for subsequent experiments. All the isolates were designated as Mp_1, Mp_2, Mp_3, Mp_4, Mp_5, Mp_6 and Mp_7. Further, the isolates were multiplied in sand maize medium (Riker and Riker, 1936) and used for further studies.

Cultural characteristics of *M. phaseolina*

Fifteen ml of the sterilized PDA medium was poured into sterile Petri dishes and allowed to solidify. A 9mm culture disc of each isolates of *M. phaseolina* was obtained from seven days old actively growing mother culture aseptically and placed at the centre of petri dishes and incubated at 28±2ºC for seven days. The radial growth and characters was observed and measured after incubation period.

With regard to sclerotial character, a 9mm culture disc of respective isolates were cut from seven days old culture and placed into 50 ml beakers separately containing 10 ml of sterile water. These beakers were kept in a mechanical shaker at 1000 rpm for 30 min. To separate the sclerotia from the medium; squeezed through cheese cloth; washed several times with distilled water and the sclerotia were transferred to a glass vial containing 2.5 ml of 2.5 percent ammonium sulphate. After 10 minutes. The floating sclerotia were filtered through a Whatmann No. 1 filter paper; rinsed with distilled water and number of sclerotia was counted using stereo zoom microscope (Dhingra and Sinclair, 1978). For each isolate 100 sclerotia were collected randomly and dried under shade for two hours and their size were measured using an ocular micrometry in a calibrated microscope.

Pathogenicity of *M. phaseolina*

The potting mixture was prepared by 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 percent level (w/w) with the sterilized soil filled in 30 cm earthen pots ten days before sowing (Sankar, 1994). Surface sterilized (using 0.1 % $HgCl_2$ solution for 30 sec. followed by two washings in sterile water) sesame seeds were sown @ 10 seeds pot^{-1}. Three replications were maintained in a completely randomized design and the sesame cultivar TMV 3 was used in this study. The pots were maintained in glass house with regular, judicious and uniform watering. The root rot incidence was recorded at 45, 60 and 75 DAS and the per cent disease incidence was calculated.

Result

Survey for the incidence of sesame root rot disease

Among the different locations of Cuddalore district surveyed for sesame root rot incidence (Table 1), Kothattai (MP_4) registered the maximum disease incidence of 34.65 percent followed by Vridhachalam (MP_7) of 32.17 percent, Bhuvanagiri (MP_2) of 28.50 percent, Palur (MP_6) of 23.75 percent and Pattampakam (MP_5) of 21.46 percent had moderate disease incidence while the minimum root rot incidence of 13.60 percent was recorded in Annamalai nagar. In general, the crop grown under rainfed conditions showed more root rot incidence when compared with the crops grown under irrigated conditions. In respect of soil type, sandy soil had more root rot incidence (15.00 to 34.65%) than clay loam (13.60 to 23.75%) soil. The results of the present survey revealed higher levels of disease incidence in rainfed crop than that of irrigated crop. The dry condition prevalent in the rainfed conditions might have favoured the pathogen which could be attributed for the higher level of disease incidence.

Table 1: Incidence of sesame root rot disease in cuddalore district.

SI. No.	Isolated place	Isolates	Variety	Soil type	Situation	Root rot incidence (%)
1.	Annamalai nagar	(MP_1)	TMV 4	Clay	Irrigated	13.60
2.	Bhuvanagiri	(MP_2)	Local	Sandy loam	Rainfed	28.50
3.	Kurinjipadi	(MP_3)	TMV 4	Red sandy	Irrigated	15.00
4.	Kothattai	(MP_4)	TMV 3	Sandy loam	Rainfed	34.65
5.	Pattambakkam	(MP_5)	TMV 4	Clay loam	Irrigated	21.46
6.	Palur	(MP_6)	Local	Clay loam	Irrigated	23.75
7.	Vridhachalam	(MP_7)	TMV 3	Red sandy	Irrigated	32.17

Cultural characteristics *M. phaseolina*

All the seven isolates of the root rot pathogen *M. phaseolina* produced

Table 2: Cultural characteristics of *M. phaseolina* native isolates.

SI. No.	Isolates	Colony character	Mycelial growth (mm)	Number of sclerotia (9mm disc)	Sclerotial size (µ)
1.	MP_1	Light grey scanty aerial growth	81.39[g]	152.49[g]	72.37[g]
2.	MP_2	grey profusely aerial growth	87.43[c]	175.69[c]	92.38[c]
3.	MP_3	Light grey scanty aerial growth	82.51[f]	158.41[f]	77.11[f]
4.	MP_4	Black profusely aerial growth	90.00[a]	188.46[a]	104.18[a]
5.	MP_5	Light grey scanty aerial growth	83.76[e]	164.72[e]	81.32[e]
6.	MP_6	Light grey scanty aerial growth	85.24[d]	171.74[d]	87.17[d]
7.	MP_7	grey profusely aerial growth	88.88[b]	179.51[b]	99.97[b]

Values not sharing a common superscript differ significantly at P < 0.05 (DMRT).

black grey profusely aerial mycelial growth on Potato Dextrose Agar (PDA) medium. Among the isolate, isolate MP_4 recorded the maximum (90 mm) mycelial growth while it was the minimum (81.39 mm) in the case of MP_1. The other isolates showed moderate mycelial growth (82.51 to 88.88 mm). Whereas, the number of sclerotial production was varying among the isolates MP_4 was found to be a most virulent recorded the maximum sclerotia of 188.46 per nine mm culture disc. While the least number of sclerotia (152.49) was produced by isolate MP_1. The rest of isolates *viz*., MP_7, MP_2, MP_6, MP_5 and MP_3 produced 179.51, 175.69, 171.74, 164.72 and 158.41 numbers of sclerotia respectively. With regard to sclerotial size, all the isolates produced varying sizes of sclerotia. While, the most virulent isolate MP_4 produced the biggest sclerotia with a size of 104.18µm and the smallest sclerotial size of 72.37µm was recorded with isolate MP_1 as the least virulent isolate. The other isolates *viz.*, MP_7, MP_2, MP_6, MP_5 and MP_3 produced sclerotia with the size 99.97µ, 92.38µ, 87.17µ, 81.32µ and 77.11µ respectively (Table 2).

Table 3: Pathogenicity of *M. phaseolina* native isolates.

SI. No.	Isolates	Root rot incidence (%)			Mean
		45 DAS	60 DAS	75 DAS	
1.	MP_1	12.25[f]	22.74[g]	24.34[g]	19.77
2.	MP_2	18.39[c]	34.61[c]	52.42[c]	35.14
3.	MP_3	15.10[d]	28.16[e]	33.12[e]	25.46
4.	MP_4	25.80[a]	49.39[a]	64.76[a]	46.65
5.	MP_5	13.75[e]	24.72[f]	28.76[f]	22.41
6.	MP_6	13.75[b]	30.82[d]	39.72[d]	28.09
7.	MP_7	24.59[b]	47.24[b]	62.56[b]	44.76

Values not sharing a common superscript differ significantly at P < 0.05 (DMRT).

Pathogenicity of *M. phaseolina* isolates

The data depicted in table 3 revealed varied levels of pathogenicity with different isolates. Among the seven isolates MP_4 was found to be more virulent with maximum incidence of 64.76 percent at 75 DAS than other isolates. This was at par with the isolate MP_7 (62.56%) collected from Vridhachalam. The isolates MP_2 and MP_6 showed 52.42 and 39.72 percent of disease incidence. The other isolates *viz*., MP_3 and MP_5, recorded 33.12 and 28.76 percent root rot incidence respectively. The least incidence (24.34%) was recorded by the isolate MP_1 collected from Annamalai Nagar. However, the isolates MP_4 and MP_7 was recorded 25.80 and 24.59% root rot incidence at 45 DAS itself.

Discussion

Bremer, (1944) also reported that dry rainfed conditions favored higher root rot disease in sesame. Dry root rot of sesame caused by *M. phaseolina* was considered as an important disease especially under rainfed conditions (Maiti *et al.,* 1985). Cruz Jimenez, (2011) observed highest *M. phaseolina* root populations in sandy soils, followed by seedlings planted in loamy sand and loam soil textures. Similar observations with *M. phaseolina* in mungbean and sesame have been reported (Hooda and Grover, 1990; Karunanithi, 1996).

Sharmishha Purkayastha *et al*., (2004) reported that the isolates of *M. phaseolina* with faster mycelial growth were found more pathogenic to cluster beans. Also, several earlier workers have reported about the variations in the mycelial growth among the isolates of *M. phaseolina* (Edraki and Banihashemi, 2010; Ijaz *et al.*, 2012). It is evident from the observations that sclerotia are the primary means of survivial (Mirza, 1984) and sufficient buildup of the growth is absolutely necessary for the aggressiveness of the pathogen. In respect of *M. phaseolina*, also, the severity of the disease is directly related to the population of viable sclerotia in the soil (Sundravadana *et al.,* 2012). Edraki and Banihashemi, (2010) who reported a sclerotia size of 85-102µ produced by *M. phaseolina*. Similar variation in the sclerotial size of *M. phaseolina* was observed by several workers (Suriachandraselvan and Seetharaman, 2004; Tandel *et al.,* 2012).

The variability in the pathogenecity among the isolates of *M. phaseolina* was reported by several workers (Byadgi and Hegde, 1985; Karunanithi, 1996). Sobti and Sharma, (1992) recorded 13 to 63 percent root rot incidence of groundnut with different isolates of *R. bataticola*. *M. phaseolina* isolated from different host species differ in their morphological and cultural characters and even differences occur in the isolates from various parts of same host (Sundravadana *et al.*, 2012). All these earlier reports were corroborate with present findings.

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