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SURVEY ON THE INCIDENCE OF COWPEA ROOT ROT DISEASE AND ASSESSING THE CULTURAL CHARACTERS AND VIRULENCE OF *MACROPHOMINA PHASEOLINA*

L. Vengadeshkumar*, M. Kalaiselvi, S. Sanjaygandhi and V. Jaiganesh

Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram -608 002, Tamilnadu, India.

Abstract

Cowpea also known as 'black eye beans' (*Vigna ungiculata L.*) is an important leguminous and hay crop in tropical and subtropical regions. In the recent years root rot caused by *Macrophomina phaseolina* causes significant losses in cowpea growing regions of Tamil Nadu. Hence, the present study was undertaken with an objective to assess the prevalence and incidence of cowpea dry root rot in different regions of Tamil Nadu, India during 2015 and assess the cultural characters and pathogenic variability among the isolates of *M. phaseolina*. The survey in different regions of Tamil Nadu revealed the endemic nature of the root rot disease with the maximum disease incidence (18.43%) recorded in Kaveripattinam of Krishnagiri region (MP_5). Also the isolates of *M. phaseolina* exhibited cultural and pathogenic variability among them the isolate MP_5 was exhibit faster mycelial growth, maximum sclerotial production and sclerotial size and recorded the maximum incidence of root rot disease under pot culture conditions.

Key words: *Macrophomina phaseolina,* Cowpea, survey, Disease incidence, Pathogenic variability

Introduction

Cowpea also known as 'black eye beans' (Vigna *ungiculata* L.) is an important leguminous and hay crop in tropical and subtropical regions, especially in the dry savanna region of West Africa (Fang *et al.,* 2007). The name "Cowpea" probably derived from when it was an important livestock feed for cows in the United States. Cowpea is called the "Hungry season crop" because it is the first crop to be harvested before the cereal crops (Gomez, 2004). Cowpea originated in Africa and South East Asia and in the southern United States (Fatokum *et al.,* 2000: Shaw and Monica, 2007) Cowpea is a good source of food, forage, fodder, vegetable and certain snacks (Nirmal *et al.,* 2001). Nigeria is the world's largest producer and consumer of cowpea, as it produces over 2.7 million tonnes of cowpea annually with an average yield of 417 kg/ha. It produces the white and brown varieties (FAO, 2015).

***Author for correspondence :** Email: vengadpragathi@gmail.com

According to FAOSTAT (2013), worldwide green pod production in 2010 was 4.5 million tonnes, intended for human consumption. Worldwide cowpeas are cultivated in approximately 8 million hectares. Area under cowpea in India is 3.9 million hectares with a production of 2.21 million tonnes with the national productivity of 683 kg/ha (Nirmal *et al.,* 2001).

Cowpea is affected by many diseases caused by viruses, bacteria, fungi and nematodes (Emechebe and Lagoke, 2002). Among the diseases, charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid is an important fungal disease that significantly reduces growth and yield in arid regions of the world (Marroni, 2015). In India, cowpea and other pulse crops are mostly cultivated under rainfed condition accounting above 78% of area and being a tropical environment, favours the disease incidence. *M. phaseolina* attacks crop plants at different stages of plant growth and causes complex disease syndromes like root rot, seedling blight, charcoal rot, ashy stem blight, wilt, collar rot, dry rot, pod rot and seed rot in

several crops (Ma *et al.,* 2010; Raguchander *et al.,* 1993).

During the recent years this disease causes significant losses in cowpea growing regions of Tamil Nadu. Hence, the present study was conducted with an objective to assess the prevalence and incidence of dry root rot of cowpea in different regions of Tamil Nadu, India during 2014-2015 and assess the cultural characters and pathogenic variability among the isolates of *M. phaseolina*.

Materials and methods

Survey for the incidence of cowpea root rot disease major cowpea growing regions in Tamil Nadu

A field survey was conducted to assess the extent of root rot incidence of cowpea in major cowpea growing regions in Tamil Nadu. Ten locations representing both rainfed and irrigated situations were selected for the study. The percent disease incidence was worked out as per phytopathometry (Marroni, 2015). Also, the infected plants showing the typical symptoms of root rot due to infection with *M. phaseolina* were collected along with rhizosphere soil for isolation of the pathogen. The other information regarding the soil type in which the crop is grown and the variety of cowpea cultivated were also recorded in the respective survey fields.

Isolation of pathogen

The pathogen *M. phaseolina* was isolated from the diseased roots of cowpea plants showing the typical root rot symptoms by tissue segment method on potato dextrose agar (PDA) medium. The axenic cultures of the different isolates of the pathogen were obtained by single hyphal tip method (Barnett and Hunter, 1972) and these were maintained on PDA slants for subsequent experiments.

Mass multiplication of *M. phaseolina* inoculum for soil application

The isolates of the pathogen were multiplied in sand maize medium (Riker and Riker, 1936) Sand and ground maize seeds were mixed in the ratio of 19:1, moistened to 50 per cent moisture content, filled in 500ml conical flask and autoclaved at 20 psi for two hours. Young actively growing mycelial discs (9mm) of the pathogen isolates were obtained from four days old culture and inoculated into each flask under aseptic condition and the flasks were incubated at room temperature (28± 2°C) for 15 days and the inoculum thus obtained was used for the experiments.

Growth and cultural characters of *M. phaseolina* isolates

Fifteen ml of the medium was poured into each of the 90 mm Petri dishes. One ml of streptomycin sulphate @ 100 ppm was added to the medium just before pouring into the plates. Inoculation was made by transferring 9mm disc of *M. phaseolina* taken from the periphery of seven days old culture. The plates were incubated at 28±2^{0}C. Mycelial growth and characters, number of sclerotia and sclerotial size were recorded.

Mycelial growth

Fifteen ml of the sterilized PDA medium was poured into sterile Petri dishes and allowed to solidify. A nine mm culture disc of *M. phaseolina* obtained from actively growing region was aseptically placed at the center of the dish and incubated at room temperature (28±2ºC). The radial growth (mm) of isolates was measured seven days after inoculation.

Sclerotial number

Four culture discs (9mm) were cut and placed into 50 ml beakers containing 10 ml of sterile water. These beakers were kept on a mechanical shaker at 1000 rpm for 30 min. to separate the sclerotia from the medium; then squeezed through cheese cloth; washed several times with dist. water and the sclerotia were transferred to a glass vial containing 2.5ml of 2.5 per cent ammonium sulphate. After 10min. the floating sclerotia were filtered through a Whatman No. 42 filter paper rinsed with dist. water and the number of sclerotia was counted using stereo zoom microscope (Dhingra and Sinclair, 1978). The number of sclerotia per microscopic field and per nine mm disc were assessed and recorded.

Sclerotial size

For each isolate 100 sclerotia were collected at random. These were dried under shade for 2h. And their size was measured using an ocular micrometer in a calibrated microscope.

Growth of *M. phaseolina* on different solid and liquid medium

Solid media

The following media were used for assessing the growth of *M. phaseolina viz.,* Potato Dextrose agar medium (Ainsworth, 1961), Oat meal agar medium (Booth, 1971), Czepek's Dox agar medium (Dox, 1910), Richard's medium (Fahmy, 1923), Peptone sucrose agar medium.

A nine mm culture disc from a 15 day old PDA culture of the pathogen was removed using a sterilized cork borer and placed at the center of sterile Petri dishes containing 20 ml of respective media under aseptic conditions. Three replications were maintained for each isolate in each medium. Seven days after incubation at

room temperature (28±2^{0}C) the mycelial growth of the isolates was assessed and recorded [Tandel *et al.,* 2012).

Liquid media

Five liquid media *viz*., potato dextrose broth, oat meal broth, Czepek's dox broth, Richard's broth and peptone sucrose broth were prepared and 100 ml of the respective medium was dispensed in 250 ml Erlenmeyer flasks, autoclaved at 1.4 kg/cm^2 for 20 min and cooled. The flask was inoculated separately with a 15 day old nine mm PDA culture disc of the *M. phaseolina*. The flasks were incubated at room temperature 28±2^{0}C for 15 days. Three replications were maintained for each medium. After incubation the mycelial mat was filtered through a pre weighed Whatman No. 1 filter paper and then dried in hot air oven at 60^{o}C till a constant weight was obtained. The mycelial dry weight was calculated by subtracting from the weight of the filter paper and recorded.

Pathogenicity test

Pots (30cm dia.) of uniform size containing sterilized soil were used for proving pathogenicity. The inoculum of *M. phaseolina* isolates multiplied in sand maize medium was mixed with soil @ 5% level ratio at the time of sowing (Sharma and Dureja, 2004). About 2 cowpea seeds were sown in each pot and maintained in green house with need based irrigation. The PDI was assessed at 30, 60 and 90 DAS and recorded. Also the plants showing the typical root rot symptom were pulled out and the pathogen was re-isolated on PDA slants. The culture thus obtained was compared with that of the original culture and the pathogenicity (Koch postulates) was proved.

Result and discussion

Survey on the incidence of cowpea root rot disease in major cowpea growing regions in Tamil Nadu

The data presented in table 1 on the roving survey conducted in some major cowpea growing regions *viz.,* Dharmapuri, Salem, Krishnagiri, Coimbatore and Chidambaram during 2014-2015 indicated the endemic nature of cowpea root rot disease. The maximum per cent disease incidence (18.43%) was recorded in Kaveripattinam of Krishnagiri region followed by Karimangalam (16.75% disease incidence) and Pennagaram (16.42% disease incidence) of Dharmapuri regions. The least incidence was recorded in Omalur of Salem region. In general root rot disease incidence was more in cultivar Paiyur-1 compared to other cultivars. Prevalence of the isolates of the pathogen differing in their virulence could be the reason for the variation in the extent of the disease incidence observed in the present study. The 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 as the reason for the higher level of disease incidence. In the present survey more root rot disease incidence was observed in black soil as compared to clay or black cotton soil. Soil texture also had a significant impact on root infections. (Cruz Jimenes, 2011) observed highest *M. phaseolina* root populations in black soils, followed by loamy sand and loam soil textures. In contrary, the crop grown in sandy loam soil registered higher per cent of root rot incidence than that of clay soil (Rettinasababady and Ramadoss, 1999).

Growth and cultural characteristics of *M. phaseolina* isolates

All the isolates of *M. phaseolina* showed morphologically similar characters with black grey profusely aerial growth and branched mycelium. Among the isolates MP_5 recorded maximum mycelial growth (90.36mm) which was followed MP_6 (89.24mm), MP_1 (87.53mm) and MP_2 (86.75mm). Also, the isolate MP_5 produced maximum number and size of sclerotia (183.52 and 105.24µ) followed by MP_6 (178.25 and 96.38µ), MP_1

Table 1: Survey for the incidence of cowpea root rot disease in different regions of Tamilnadu 2014-2015.

Isolates no.	Districts	Village	Soil type	Variety	Situation	Root rot incidence (%)
MP_1	Dharmapuri	Karimangalam	Black soil	Paiyur-1	Rainfed	16.75^b
MP_2		Pennagaram	Black soil	Paiyur-1	Rainfed	16.42^b
MP_3	Salem	Omalur	Red	CO2	Rainfed	12.38^f
MP_4		Mettur	Black soil	Paiyur-1	Irrigated	12.88^f
MP_5	Krishnagiri	Kaveripattinam	Black soil	Paiyur-1	Rainfed	18.43^a
MP_6		Pochampalli	Black soil	Paiyur-1	Rainfed	16.41^b
MP_7	Coimbatore	Singanallur	Black cotton soil	CO2	Rainfed	13.75^e
MP_8		TNAU	Black cotton soil	CO6	Irrigated	12.48^f
MP_9	Chidambaram	Sivapuri	Clay	Local variety	Rainfed	15.82^c
MP_{10}		Virudhachalam	Clay	Local variety	Rainfed	14.48^d

Table 2: Growth and cultural characters of *M.phaseolina* isolates

Isolates	Mycelial growth (mm)	Mycelium characters	No. of sclerotia (9mm disc)	Sclerotial size (µ)
MP_1	87.53[c]	Light grey scanty aerial growth	169.57[c]	9.71[c]
MP_2	86.75[d]	Black scanty aerial growth	168.73[c]	84.25[d]
MP_3	78.60[i]	Black grey profusely aerial growth	156.25[g]	79.45[h]
MP_4	80.65[h]	Black scanty aerial growth	158.75[f]	80.10[g]
MP_5	90.36[a]	Black grey profusely aerial growth	183.52[a]	105.24[a]
MP_6	89.24[b]	Black grey profusely aerial growth	178.25[b]	96.38[b]
MP_7	8375[f]	Black grey profusely aerial growth	160.50[e]	80.90[g]
MP_8	82.50[g]	Black grey profusely aerial growth	160.00[e]	80.38[g]
MP_9	84.50[e]	White grey profusely aerial growth	164.75[d]	83.70[e]
MP_{10}	84.25[e]	Grey profusely aerial growth	162.50[d]	82.50[f]

(169.57 and 89.71µ) and MP_2 (168.73 and 84.25µ). The least growth, minimum number and size of sclerotia were observed with the isolate MP_3 (78.63mm, 156.25 and 79.45µ) (Table 2). *M. phaseolina* isolates from pearl millet, sesame, horse gram, maize and moth bean differed in their mycelial growth.

Showed marked variation in cultural characters (Sharma and Dureja 2004; Shekhar *et al.,* 2006). Similar such variation in the cultural characteristics of *M. phaseolina* on PDA was reported by Tandel, *et al.* (2012). 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 a1.,* 2012). With regard to number and size of sclerotia, all the isolates of *M. phaseolina* varied in their ability to produce sclerotial population and size on PDA medium. The maximum sclerotial number and size with 183.52 and 105.24 µ was obtained from MP_5 which was also showed most virulent isolate. The minimum number and size of sclerotia with 156.25 and 79.45 µ was recorded by MP_3 the least virulent isolate (Table 2). Various reports was revealed the variations in the number and size of sclerotia of *M. phaseolina* (Sharmisha *et al.,* 2004; Suriachandraselvan and Seetharaman, 2003; Umer Iqbal *et al.,* 2010).

Growth of *M. phaseolina* (MP_5) on different solid and liquid media

Among the media tested, Potato dextrose agar and broth were found to be the best in supporting the mycelial growth and dry weight of *M. phaseolina* as they recorded significantly the maximum mycelial growth and dry weight (90.00 mm and 310.54 mg) followed by Oatmeal agar medium (83.53 mm and 289.17 mg) and peptone sucrose agar medium (82.35 mm and 224.72 mg) in the decreasing order of merit. The least growth and dry weight of 75.76 mm and 196.63 mg was observed in Richard's agar medium (table 3). Similarly Sayyad, *et al.* (2015) reported that PDA medium as the best for the growth of *M. phaseolina*. The variation on the growth of *M. phaseolina* in different media was reported by earlier workers (Shaw and Monica, 2007; Muthukumar *et al.,* 2014). All these earlier reports corroborate with the present findings.

Pathogenecity of *M. phaseolina* isolates

The data pertaining to the pathogenecity of *M. phaseolina* isolates is presented in table 4. Among the ten isolates tested, isolate

Table 3: Growth of *M. phaseolina* (MP_5) on different solid and liquid medium.

S. No.	Media	Growth of *M. phaseolina* MP_5	
		Solid media (mm)	Liquid media (mg)
1	Potato dextrose agar	90.00[a]	310.54[a]
2	Oat meal agar	83.53[b]	289.17[b]
3	Czapek's dox agar	78.46[d]	265.88[c]
4	Richard's agar	75.76[e]	196.63[c]
5	Peptone sucrose agar	82.35[c]	224.72[c]

MP_5 was found as the most virulent which recorded maximum mean per cent disease incidence (31.75%) on cowpea variety Paiyur-1 which was followed by MP_6 with a PDI of 27.74 per cent, MP_1 with a PDI of 26.69 per cent and MP_2 with a PDI of 26.55 per cent. The least PDI (17.12%) was recorded with isolate MP_3. The virulent culture MP_5 exhibited brown to black lesion on stem with in twenty days after inoculation. The leaves also showed drying appearance. To confirm the pathogenicity, the pathogen was reisolated and its characters were studied and compared with original

Table 4: Pathogenicity of *M. phaseolina* isolates

S. No.	Isolates	Root rot incidence (%)			Mean (%)
		30 DAS	60DAS	90 DAS	
1	MP_1	20.80[c]	28.63[b]	33.62[c]	26.69
2	MP_2	18.42[d]	28.32[b]	34.24[b]	26.55
3	MP_3	12.48[g]	18.46[g]	20.43[g]	17.12
4	MP_4	12.26[g]	22.24[f]	28.18[f]	20.89
5	MP_5	25.92[a]	30.71[a]	38.64[a]	31.75
6	MP_6	21.09[b]	27.75[c]	34.37[b]	27.74
7	MP_7	15.74[e]	24.62[e]	31.74[d]	24.03
8	MP_8	14.98[f]	22.76[f]	29.72[e]	22.48
9	MP_9	18.44[d]	28.10[b]	33.53[c]	27.68
10	MP_{10}	18.24[d]	26.88[d]	34.54[b]	26.99

culture. Therefore, MP_5 the most virulent isolate was used throughout the study. The variations in root rot incidence in different locations could be well attributed to the difference in virulence of the *M. phaseolina* isolates prevalent in the respective areas. In a similar study, Ratnoo, *et al.* (1997) reported that variation in the isolates of *M. phaseolina* from different cowpea growing areas of Udaipur.

The variability in the ability of virulence among the isolates of *M. phaseolina* have been also reported by earlier workers (Su *et al.,* 2001; Barnett and Hunter, 1972; Rayatpanah and Dalili, 2012). The above reports are in agreement with the present investigation. Besides an increase in the root rot incidence was observed with an increase in the age of the crop.

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