

Variability in Chickpea Rot-causing Soil-borne Necrotrophs, Sclerotium rolfsii and Macrophomina phaseolina

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ABSTRACT

The present work was designed to identify the cultural and pathogenic variability of the two chickpea rot-causing necrotrophic soil-borne pathogens i.e. *Sclerotium rolfsii* and *Macrophomina phaseolina* cause significant damage to chickpea cultivation. The potentiality of the isolates for infection was recognized with artificial inoculation test using susceptible genotypes. Disease index values of *S. rolfsii* and *M. phaseolina* were 24.9–68.8% and 20.0–64.0%, respectively. Among twelve isolates of *S. rolfsii*, BAUSr4 and Ag2 produced the highest infection on genotype L550 (cd: 10.79). Likewise, isolate DarkMP4J followed by DarkMP1J and Jute1, among twenty—one isolates of *M. phaseolina*, rendered maximum infection on genotype K850 (cd: 5.15). No relationship was established among the cultural characters and pathogenicity of the isolates. Isolates differed in aggressiveness across different locations and hosts.

Keywords : Chickpea rot, *Macrophomina phaseolina*, necrotrophs, *Sclerotium rolfsii*, soil-borne pathogen

INTRODUCTION

Chickpea (Cicer arietinum) is one of the oldest pulse crops cultivated in India. It is cultivated across the world but over 85% of chickpea is being cultivated in Asia. India shares the largest chickpea production (65%) of global production. Presently, in Bihar, it is grown in 0.6 m ha area and the production has drastically reduced due to a rapid decline in the cultivating area. In Bihar, chickpea is cultivated with very low input and therefore the production remains very low as a response. Once chickpea was the most important pulse crop but at present, it is most affected by the change in cropping preference of farmers, reasonably infestation of insects and disease problems. The occurrence of diseases deters farmers to grow chickpea. Due to recurrence of diseases, the farmers are shifting from chickpea to lentil cultivation. Considering the pathogenic problems more than 50 diseases have so far been reported on chickpea; among them, the soil-borne problems including Fusariumwilt, charcoal rot, damping-off, southern blight (collar rot) and black root rot are most dreaded. Among necrotrophic soil-borne pathogen, Sclerotium rolfsii and Macrophomina phaseolina are emerging as a major threat for chickpea production worldwide (Nene et al., 1996), in India (Ghosh et al., 2013) and in Bihar (Sultana et al.,2016). Yield loss in chickpea production due to S. rolfsii ranges 10-30% (Maurya et al., 2008) whereas due to M. phaseolinathe production may decline to 10-20% (Manjunatha and Saifulla, 2016).

These pathogens have become more important in recent years due to drastic climate change which makes the pathogen more aggressive and increased with adaptability to the environment (Ghatak and Ansar, 2017; Kumar et al., 2017; Savary *et al.*, 2010). The destructive stem and root disease of

southern blight and charcoal rot considered as major soil-borne diseases of chickpea caused by necrotrophic pathogens, S. rolfsii and M. phaseolina (Ghosh et al., 2013). S. rolfsii typically produces abundant white mycelium and small, brown, round sclerotia on the diseased tissue under hot humid conditions, and may spread over the soil surface from a nutrient base such as a diseased stem base, diseased pods and leaf residue (Songvilay et al., 2012). The prevalence of charcoal rot disease can be enhanced by different physiological and ecological factors such as low moisture contents, high temperature, heat and the stress associated with host reproduction (Gade et al., 2018). M. phaseolina produces minute black sclerotia which cause rotting of tissue to become blackened and most often seen during summer weather (Gulya et al., 2002). Microsclerotia viability of M. phaseolina declines under high soil moisture and flooded condition compared to dry soils (Pratt, 2006). The north Indian state Bihar has two extreme types of topography; therefore, this area provides an ideal condition to study on the variability of these soil-borne necrotrophs that may generate the information in identifying the pattern of virulence of S. rolfsii and M. phaseolina and help to set the management strategies.

These necrotrophs survive using the asexual mode of reproduction and the ability to infect many hosts (Su *et al.*, 2001; Xie *et al.*, 2014). Both of these necrotrophs overwinters as mycelium or sclerotia (or microsclerotia) in debris or plant tissues or soil. Moreover, thesesoil–borne fungi are difficult to manage by applying physical and cultural methods due to its wider infection range of plant species. To counter the problems associated with necrotrophic soil–borne pathogens, present work was designed to observe the pathogenic variability of the two necrotrophic fungi. Pathogenicity of twelve isolates of *S. rolfsii* and twenty–one isolates of *M*.

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phaseolina collected from different locations of Bihar over a range of hosts were analyzed on the susceptible genotype of chickpea for each pathogen.

A better understanding of variability among *S. rolfsii* and *M. phaseolina*isolates from different locations of Bihar and host will assist breeders in the optimization of breeding studies toenable long–term resistance for different geographical origin and host. Therefore, the objectives of this study were(i) to evaluate the cultural variability among the two soil–borne chickpea–infecting necrotrophs, and (ii) to assess the pathogenic variability of isolates of *S. rolfsii* and *M. phaseolina* collected from different locations and host plants.

MATERIAL AND METHODS Collection of fungal isolates

Diseased plants exhibiting characteristic symptoms of southern blight or charcoalrot were collected from different fields and brought to the laboratory at Bihar Agricultural University, Sabour for isolation. Upon arrival, the disease samples collected from different locations of Bihar were stored in a refrigerator (4°C) for 1–2 days. Infected stalk, seed, root or collar region was used for isolation. Some isolates of these fungi were obtained from the Plant Pathology repository. The isolates used in this study were presented in (Tables 1 and 2).

Table 1: Origin and characteristics of various isolates of Sclerotium rolfsii

Isolate		Origin		Year of		
Number	Code	Host	Location	collection	Mycelium type	
1	BAUSr4	Cucumber	Sabour	2016	Fluffy	
2	Ag2	Ash gourd	Sabour	2017	Fluffy	
3	Ag3	Ash gourd	Sabour	2017	Fluffy	
4	Ag5	Ash gourd	Sabour	2017	Fluffy	
5	Bg3	Bottle gourd	Patna	2017	Fluffy	
6	Bg4	Bottle gourd	Patna	2017	Fluffy	
7	Bg5	Bottle gourd	Patna	2017	Fluffy	
8	Bg6	Bottle gourd	Patna	2017	Fluffy	
9	BAUSr7	Bitter gourd	Sabour	2016	Compact	
10	BAUSr10	Brinjal	Sabour	2016	Compact	
11	BAUSr9	Lentil	Sabour	2016	Fluffy	
12	BAUSr13	Lentil	Naugachia	2016	Fluffy	

Table 2: Origin and characteristics of various isolates of Macrophomina phaseolina

Isolate		Origin		Year of	Mycelium	Colony
Number	Code	Host	Location	collection	type	colour
1	J1Grey	Jute	Katihar	2015	Appressed	Grey
2	J2Grey	Jute	Katihar	2015	Appressed	Grey
3	J3Grey	Jute	Katihar	2015	Appressed	Grey
4	Jute1	Jute	Katihar	2016	Appressed	Black
5	Jute2	Jute	Katihar	2016	Appressed	Black
6	Jute3	Jute	Katihar	2016	Appressed	Black
7	Jute4	Jute	Katihar	2016	Appressed	Black
8	Jute5	Jute	Katihar	2016	Fluffy	Black
9	Jute6	Jute	Katihar	2016	Fluffy	Black
10	Jute7	Jute	Katihar	2016	Appressed	Black
11	Jute8	Jute	Katihar	2016	Appressed	Black
12	Jute9	Jute	Katihar	2016	Appressed	Black
13	Jute12	Jute	Katihar	2016	Appressed	Black
14	Jdark1	Jute	Katihar	2017	Appressed	Black
15	Jdark2	Jute	Katihar	2017	Appressed	Black
16	Jdark3	Jute	Katihar	2017	Appressed	Black
17	CP3	Cowpea	Patna	2017	Velvetty	Black
18	DarkMP1J	Jute	Katihar	2017	Fluffy	Black
19	DarkMP2J	Jute	Katihar	2017	Fluffy	Black
20	DarkMP4J	Jute	Katihar	2017	Fluffy	Black
21	WhiteMP1J	Jute	Katihar	2017	Fluffy	White

Fungus isolation

About 15–20 ml of potato dextrose agar (PDA) medium, supplemented with streptomycin sulphate, was pouredin each Petriplate. The infected samples (~5 mm size) were treated with 1% NaOCl for 30 seconds followed by washing in sterilized distilled water (SDW) for successively three times to remove NaOCl solution. These surface sterilized bits of infected stalk, seed, root, and collar region was placed in Petriplate containing PDA. The inoculated Petriplates were incubated at 25±2°C and 28±2°C for *S. rolfsii* and *M. phaseolina*, respectively. After 2–3 days, sub-culturing was done on PDA slants and allowed to incubate for 6–7 days as above stated temperatures for the respective fungi. These purified slants with active mycelium of each pathogen were stored in refrigerator at 4°C and used whenever required.

Infection of Sclerotium rolfsii and Macrophomina phaseolina

Pathogenicity of the sampledisolates of Sclerotium rolfsii was

tested by soil infection method(Sahni et al., 2008). The sterilized sandy-loam soil mixed with mass multiplied culture of S. rolfsii @1000 sclerotia/kg soil (Fig.1). Seeds of chickpea (genotype L550) were surface sterilized with 1% NaOCl for 1 min followed by three successive rinsing with sterile water. Seeds were sown in the inoculated soil following completely randomized design (CRD). Observations on germination, pre- and post-emergence mortality were recorded. Soil without test fungus was treated as control. Pathogenicity of Macrophomina phaseolina isolates were tested by blotter paper technique (Nene et al.,1981), and presented in Fig. 2. Seedlings (genotype K850) inoculated with a suspension prepared with mycelial mat of M. phaseolina. The seedlings were dipped in the suspension for 5 mins and then wrapped in the wet blotter paper. The seedlings without inoculation were used as control and treated with sterile water. The assessment made after 5-6 days of inoculation.



Fig. 1: Screening of chickpea genotype to Sclerotium rolfsii



Fig. 2: Screening of chickpea genotype to Macrophomina phaseolina

Experimental design, disease assessment and data analysis

The mean values of pathogenicity and frequencies of reactions of resistance/ susceptibility caused by each isolate were calculated. Mean values were subjected to analysis of variance following complete randomized design (CRD) and were separated by the critical difference at P=0.05, where the effect of variation among the isolates in disease development were identified. Disease severity caused by each isolate (of different fungi) on chickpea genotypes was assessed by using the different disease rating scales (1–5). Rating scale of Sclerotium rolfsii developed by Le et al. (2012): 1 = no disease symptom; 2 = disease symptoms without visible fungal outgrowth; 3 = disease symptoms with visible fungal outgrowth; 4 = partial wilting of plant; and 5 = complete wilting and death. The rating scale of Macrophomina phaseolina developed by Abawi and Pastor-Corrales (Igbal et al., 2010) was followed in this experiment: 1 = no discoloration and no microsclerotia visible; 2 = no discoloration of vascular tissue, with very few

microsclerotia visible in the pith, vascular tissue or under the epidermis; 3 = partially discolored vascular tissue, with microsclerotia partially covering the tissue; 4 = discoloured vascular tissue, with numerous microsclerotia visible in the tissue under the outer epidermis, in stem and root sections; and 5 = vascular tissue with numerous microsclerotia producing a dark color inside and outside of the stem and root. Analysis was performed using statistical software STPR package.

RESULTS AND DISCUSSION

${\bf Cultural\ variability\ in\ } {\it Sclerotium\ rolfsii}\ {\bf and\ } {\it Macrophomina\ } {\it phaseolina}$

Significant variability was observed among twelve isolates of *Sclerotium rolfsii* and twenty–one isolates of *Macrophomina phaseolina*. Assessments were made on the origin (host and location) of the isolates and their mycelium type (Tables 1 and 2). The isolates of *S. rolfsii* produced two types of

mycelium*viz.*, compact and fluffywith white colony colour (Fig. 3).Maji *et al.* (2018) observed white mycelium growth of *S. rolfsii* isolates on PDA medium. They also reported the

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white colour mycelium seen on the infected tissue of wheat and over the soil surface. The fungus perpetuates as sclerotia on plant debris and in soil (Cilliers *et al.*, 2000).



Fig. 3: Colony characteristics of Sclerotium rolfsii isolates used in this study; (a) compact, and (b) fluffy

Among mycelium type, only two isolates of *S. rolfsii* (BAUSr7 and BAUSr10) showed compact colonies and the rest ten isolates produced fluffy colonies. Maji *et al.* (2018) reported variation in isolates of *S. rolfsii* on parameter tested for mycelium growth, colony appears and colony colour in which out of nine isolates, colonies of five isolates were fluffy, whereas 4 were compact. The similar result reported by Prasad *et al.* (2012) for isolates of *S. rolfsii* on the basis of cultural characters and pathogenic variability.

The *M. phaseolina* isolates produced three types of colonies *viz.*, white, grey and black(Fig. 4). One isolate (WhiteMPIJ) showed a white colonyand three isolates (J1Grey, J2Grey, and J3Grey) expressed grey colony while the rest seventeenisolates exhibited a black colony. Moreover,

theseisolates rendered three types of colonies *viz.*, fluffy, appressed and velvety. The isolate obtained from cowpea (CP3) showed velvety colony, whereas six isolates*viz.*, Jute5, Jute6, DarkMP1J, DarkMP2J, DarkMP3J, and WhiteMP1J showed fluffy colony, and the rest fourteen isolates were found with the appressed colony. Gupta *et al.* (2012) observedgrey to black colonies of *M. phaseolina* develop on the medium. Smitha *et al.* (2016) reported similar result based on morphology, the isolates were grouped into two cultural categories *viz.*, isolates with appressed type growth and fluffy type growth which demonstrated the existence of variability within *M. phaseolina* isolates causing pigeonpea root rot. Aghakhani and Dubey (2009) isolated *M. phaseolina* from root rot infected chickpea plants and reported variations in colony colour from white to black.



Fig. 4: Colony characteristics of Macrophomina phaseolina isolates used in this study; (a) fluffy, (b) appressed, and (c) velvety

Making categories of the isolates often provide their pathogenic identification correlated with the physical appearance of an isolated while cultivated on a medium. Several workers grouped the *S. rolfsii* and *M. phaseolina* isolates associated with diverse crops into different categories based on colony characters (Sarma et al., 2002; Sharma et al., 2012 and Datta et al., 2013). These results reveal wide variation among isolates of *S. rolfsii* and *M. phaseolina* in cultural characteristics which could be due to differences in nutritional requirement and genetic characteristics as suggested for the pathogen of rice blast and finger millet blast (Balodi et al., 2015).

Cultural characteristics such as mycelium type and colony colour can also be used to distinguish isolates of these two pathogens, although the work does not demonstrate that relationships exist among cultural character and aggressiveness of isolates. A positive correlation between cultural character, geographical location, and aggressiveness of S. rolfsii isolates has been detected (Kumar 2017 and Xie et al., 2014). Study of cultural character of isolates could be effectively used to determine the nutritional requirement of isolates from the different geographical location. Utilization of nutritional requirements also explains the pathogenic importance of a soil-borne fungal pathogen, Therefore, identification of relationship among cultural characters of isolates and their pathogenicity would be the key point for breeding purpose in chickpea genotype against these pathogens.

Pathogenic variability of Sclerotium rolfsii and Macrophomina phaseolina

Pathogenicity of twelve isolates of *Sclerotium rolfsii* and twenty–one isolates of *Macrophomina phaseolina* was tested by artificial inoculation on respective susceptible genotypes of chickpea (Figs. 5 and 6). The present experiment was conducted in laboratory condition in which pathogens were artificially inoculated on susceptible genotype of chickpea where for *S. rolfsii*, L550 and for *M. phaseolina*, K850 was selected.

Disease assessment for each pathogen was done on the basis of 1–5 disease rating scale given by Le et al. (2012) and Iqbal et al. (2010) for S. rolfsii and M. phaseolina, respectively. The in-planta screening was done as per the standard procedure given is by Sahni et al. (2008) for S. rolfsii and Nene et al. (1981) for M. phaseolina. Yaqub and Shahzad (2005) demonstrated the pathogenic variability of S. rolfsii isolates on sunflower, mungbean, sugar beet, tomato and lentil by artificial inoculation following soil infection method. In this study, the artificial infection produced considerable infection on roots of S. rolfsii and M. phaseolina. Disease index ranged between 24.9-68.8% and 20.0-64.0% for S. rolfsii and M. phaseolina, respectively (Figs. 5 and 6). Curtis et al. (2010) observed disease index of S. rolfsii71.4-100.0% on the susceptible genotype of tomato. However, for M. phaseolina isolates, Rayatpanah et al. (2014) observed disease index 19.0-24.0% and 27.0-30.0% for sunflower and soybean, respectively. Our results indicate pathogenic variability exists

among the soil-borne necrotrophs, *S. rolfsii* and *M. phaseolina* in Bihar.

Among twelve isolates of *S. rolfsii*, BAUSr4 isolate obtained from host cucumber and location Sabour showed highly aggressiveness as compared to other isolates (Fig.5); furthermore, the isolate Ag2 obtained from ash–gourd at Sabour was *at par* to BAUSr4 collected from cucumber at Sabour (cd: 10.79). Isolates BAUSr9 obtained from lentil at Sabour and BAUSr13 collected at Naugachia from lentil were least aggressive among other isolates, whereas Ag3, Ag5, Bg3, Bg4, Bg5, Bg6, BAUSr7, and BAUSr10 were moderately aggressive.

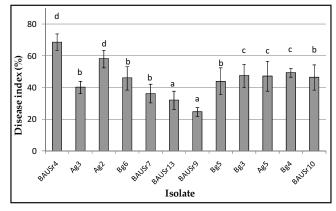


Fig. 5: Disease index of various isolates of *Sclerotium rolfsii* on chickpea susceptible genotype L550. Means followed by different letters over the columns are significantly different (cd: 10.79). Error bars are standard error of the means.

The comparison amongst twenty–one isolates of M. phaseolina is presented in Fig. 6.Isolate DarkMP4J obtained from Jute at Katihar showed high aggressiveness followed by DarkMP1J (cd: 5.15). Under least aggressiveness category isolates CP3, Jute6, Jute8, Jute12, J1Grey, J2Grey, DarkMP2J, WhiteMP1J, JDark1 and JDark2 were identified. Likewise, isolates Jute1, Jute2, Jute3, Jute4, Jute5, Jute7, Jute9, J3Greyand JDark3 were showed the reaction of moderate aggressiveness. In our case, there was also no significant correlation between the isolates aggressiveness and their geographical or host plant origin. In a random selection, not all the identified highly aggressive isolates had been isolated from the same host and location on the tested chickpea genotype. These results were in agreement with the results of Flores-Moctezuma et al. (2006), Le et al. (2012), Omar et al. (2007), and Awasthi et al. (2010). However, Xie et al. (2014) demonstrated the isolates of the same location always exhibited the highest level of disease severity compared with the other isolates, regardless of the host plant.

This investigation advocates for understanding the behaviour of host and pathogen in the process of disease development. Host–pathogen relationships are crucial for reliable breeding program for disease resistance (Barchenger et al., 2018; Pagán and García-Arenal, 2018). Developing resistance against these pathogens in the chickpea genotypes would provide a cost–effective and environmentally safe method for managing

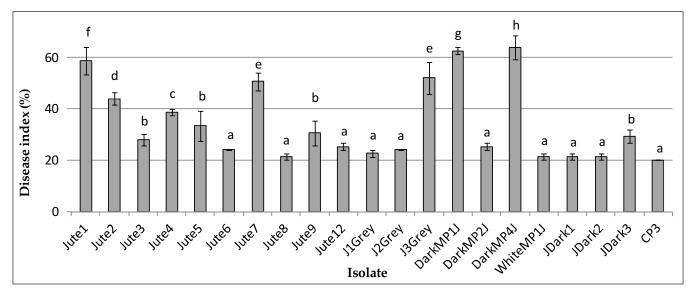


Fig. 6: Disease index of various isolates of *Macrophomina phaseolina* on chickpea susceptible genotype K850. Means followed by different letters over the columns are significantly different (cd: 5.15). Error bars are standard error of the means.

these diseases. Such information on variability within populations in the geographic region contributes to growing knowledge of biology and epidemiology of these economically important pathogens and assists the development of effective control strategy. It is quite evident that variability of pathogens in cultural, morphological and pathogenic parameters is imperative for the pathogen to have a better adaptation in response to diversified environmental factors (Ghatak and Ansar, 2017). This would further lead to host–plant resistance, development of resistant varieties of different crops against diseases, and implementation of new disease management strategies.

CONCLUSION

In the present study a considerable diversity elucidated in the population of *S. rolfsii* and *M. phaseolina* collected from Bihar. It suggests fortheir ability to adapt in diverse conditions and to overcome the host resistance. The isolates of *S. rolfsii* and *M. phaseolina* were showed the cultural variability on PDA plates and pathogenic variability on susceptible chickpea genotypes.

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These results will be useful in developing integrated strategies for management of chickpea against southern blight and charcoal rot and breeding programs for chickpea and other crops affected by these pathogens. The determination of variability among isolates of *S. rolfsii* and *M. phaseolina* is elementary for development of disease management strategies for different geographical regions.

The variability in isolates obtained from cultural and pathogenic tests may be considered an important parameter for policy development in disease management systems. Also, the results will be useful in breeding programmes of chickpea genotypes resistant to southern blight and charcoal rot.

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