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Screening of mungbean genotypes against leaf blight pathogen *Macrophomina phaseolina* (Tassi) goid

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Abstract
Leaf Blight of Mungbean caused by *Macrophomina phaseolina* (Tassi) Goid is one of the more severe yield destabilizing factors causing serious yield losses each year in Central India. Mungbean was observed severely affected by leaf blight in *Kharif* as well as during summer season. A total of 52 germplasm lines of mungbean were screened against *Macrophomina* blight at Pulse Pathology Field, Block Number-23 of University Research Farm, Raipur with two replications each. Per cent incidence was recorded on the basis of visual observation according to 1-5 scale given by IIPR, Kanpur. Out of 52 entries of mungbean screened for *Macrophomina* blight under natural field conditions, 21 cultivar found resistant, 16 cultivar found moderately resistant, 5 cultivar found moderately susceptible, 9 cultivar found susceptible and KPM 16-50 were found highly susceptible whereas KPM 16-50 used as susceptible check in sick plot of these test lines.

Keywords: Mungbean, *Macrophomina* blight, screening, genotypes, germplasm

Introduction
The mung bean [*Vigna radiata* (L.) R. Wilczek] is a legume cultivated for its edible seeds and sprouts across the Asia. There are 3 subgroups of *Vigna radiata*: one is cultivated (*Vigna radiata* subsp. *radiata*), and other two are wild (*Vigna radiata* subsp. *sublobata* and *Vigna radiata* subsp. *glabra*). The total area covered under moong in India was 30.530 lakh hectares with a total production of 15.087 lakh tones having productivity of 494 kgha^{-1}. The coverage of area and its production was maximum in Rajasthan i.e. 8.975 lakh hectares and 4.645 lakh tones respectively. The highest yield was recorded by the state of Punjab (853 kgha^{-1}). The National yield average was 494 kgha^{-1}. (Anonymous, 2016) [1], the total area covered under mung in Chhattisgarh was 0.149 lakh hectares with a total production of 0.039 lakh tones having productivity of 262 kgha^{-1}. (Anonymous, 2016) [1]. Throughout the India, the mungbean is used for different purposes. The major portion is utilized in making dal, curries, soup, sweets and snacks. With sprouting there is an increase in the thiamine, niacin and ascorbic acid, thus mungbean sprouts are increasingly becoming popular in certain vegetarian diets. Moreover, its food values lie in high and easily digestible protein. The grains contain approximately 25-28% protein, 1.0-1.5% oil, 3.5–4.5% fiber, 4.5–5.5% ash and 62–65% carbohydrates on dry weight basis. Amino acid analysis indicates that it is an excellent complement to rice for balanced human nutrition. The major fungal diseases which infect the crop are leaf blight *[Macrophomina phaseolina* (Tassi) Goid], powdery mildew (*Erysiphe polygoni* DC), web blight (*Rhizoctonia solani* Kuhn), *Cercospora* leaf spots (*Cercospora canescens* Ellis and Martin, *C. cruenta* Sacc., *C. dolichi* Ellis and Everlast, *C. kikuchi* Matsumoto & Tomoyasu and Anthracnose (*Colletotrichum dematium and C. lindemuthianum* (Philip *et al*., 1969, Dwivedi and Saksena, 1974., and Grewal,1988) [5, 6].

Macrophomina phaseolina (Tassi) Goid is one of the most damaging seed and soil borne pathogen, infecting about 500 plant species in more than 100 families throughout the world [(Kunwar *et al,* 1986, Mihail and Taylor 1995)] [8, 9]. Under favourable conditions the fungus causes many diseases like leaf blight, damping off, seedling blight, collar rot, stem rot, charcoal rot and root rot in various economically important crops. Mungbean was observed severely affected by leaf blight caused by *Macrophomina phaseolina* (Tassi.) Goid. in *Kharif* as well as during summer season. It was first reported from Jabalpur (M.P.) India (Philip *et al*., 1969).

The pathogen attacks on all parts of plant i.e. root, stem, branches, petioles, leaves, pods and seeds. Moreover, seed infection of *Rhizoctonia bataticola* (*M. phaseolina*) ranges from 2.2-15.7% which causes 10.8% in grain yield and 12.3% in protein content of seed in mungbean (Kaushik *et al.*1987). The infected seeds act as an important source of primary inoculum for new areas. Soil and seed borne nature of the disease possesses problems for an effective disease management. Therefore, an attempt has been made to integrate management of leaf blight disease on mungbean incited by *Macrophomina phaseolina* (Tassi) Goid which have become a serious problem in hampering the production of the mungbean in all growing areas of India.

Now-a-days fungicides have shown resistance against some diseases in India. It has also drastic effects on the soil as well as environment. Due its high cost, farmers are reluctant to apply it in the field. So here come the era of resistant varieties. Disease resistant varieties are advantageous as they do not get infested with pathogens. Their genes are modified so pathogens cannot harm them. As pathogens cannot harm them so fungicides are not used to protect which in turn save environment from pollution caused by fungicides. So, it is imperative to identify the source(s) of its resistance and exploit it to develop resistant varieties of mungbean through breeding approaches. Mainly, Field screening are used to screen mungbean genotypes for leaf blight resistance.

Materials and Methods

Collection of disease sample

The disease samples of mungbean having dark brown irregular lesions were collected from the field of Pulse Pathology Research Farm, I.G.K.V., Raipur.

Isolation, purification and maintenance of culture and identification of pathogen

The isolation of pathogen was made from the disease-infected leaf and stem collected from the field of Pulse Pathology Research Farm. The usual tissue isolation method was followed for the isolation of the fungus from leaves, infected branches and stem. Infected leaf bits of mungbean were first washed with tap water and then with distilled water. The bits were then surface sterilized by dipping in 1% sodium hypochlorite solution for a minute and again washed by giving three successive changes of sterilized distilled water to remove the traces of Sodium hypochlorite. The isolation work was carried out by using laminar airflow. The leaf and stem bits were then placed on sterilized potato dextrose agar in petri plates. Plates were then incubated at room temperature (28±2 °C). As soon as the growth of fungus was observed in plates, small portion of mycelial growth was transferred on potato dextrose agar slants. Numbers of slants were prepared for further investigation. Two per cent Water Agar and Potato Dextrose Agar were used for isolation, purification, maintenance and morpho-cultural studies of isolates. The fungus isolated was purified by repeated isolation from the culture plates. The pathogen was identified on the basis of character of the mycelium and sclerotia. The characters were compared with the standard description of *Macrophomina phaseolina* from literature (Singh R.S, 1998) [11].

Preparation of inoculum of the pathogen

The pathogenic strain of *M. phaseolina* isolated from diseased roots of mungbean was multiplied on sorghum grains. The grains were at first soaked in water overnight, washed and half-boiled in water and filled in 250 ml conical flasks to 1/4th of their capacity and sterilized at 15 lbs pressure for 15 minutes. Thereafter, *M. phaseolina* was inoculated in the flasks and incubated at 28º C for 15 days. The flasks were shaken every day thereafter.

Screening of the genotypes

To examine the sources of resistance against *M. phaseolina* fifty two mungbean genotypes were screened under field condition at Pulse Pathology Field, Block Number-23 of I.G.K.V. Raipur Research Farm during *Kharif* 2016-17 following standard agronomical practices. After multiplication, The inoculum was mixed in soil at the rate of 200 gm per row of 3m, one week prior of sowing at 5-10 cm depth. The each genotype was sown in two replications with two rows of 3 m length. Observations were recorded after 15, 30 and 45 days of sowing. The genotypes were categorized according to their disease reaction based on 1-5 scale suggested by IIPR, Kanpur.

Table 1: Categorization of entries on the basis of per cent disease incidence and 1-5 scale given by IIPR, Kanpur

S. No.	Score	PDI	Category
1	1	0	Resistant
2	2	0.1-10.0	Moderately resistant
3	3	10.1-25.0	Moderately susceptible
4	4	25.1-50.0	Susceptible
5	5	Above 50.0	Highly susceptible

Results and Discussion

The *Macrophomina* blight disease samples of leaf and stem were collected from Thirteen different areas in raipur and subjected to isolation of the causal organism. Two disease samples from stem and two samples from leaf of each variety were collected. *Macrophomina phaseolina* could only be isolated from leaf samples of all the varieties because the pathogen was observed to be more pre-dominant on leaf as compared to the stem. Therefore, the pathogen isolated from leaf was used in further investigation. Isolation of the fungus was done in Petri dishes using Potato Dextrose Agar medium. The surface sterilized diseased bits yielded the fungus after 48 hours of incubation at 28 ±2 °C temperature. The uniform colonies originating from diseased bits were separated, purified and were maintained on potato dextrose agar slants for further use during entire course of investigation. The fungus isolated was purified by repeated isolation from the culture plates. The pathogen was identified on the basis of character of the mycelium and sclerotia. The mycelium was septate and hyaline, the sclerotia were brown to black in colour, rounded or oblong in shape. Numerous black sclerotia were produced within 5 to 6 days on Potato Dextrose Agar medium. Pathogenicity test was performed and fungus isolated was confirmed as *Macrophomina phaseolina*.

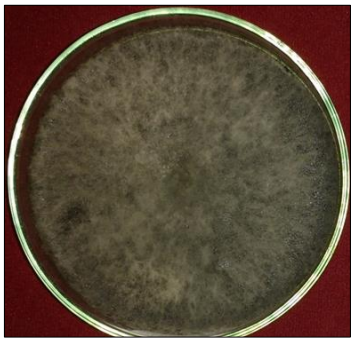

Plate 1: Pure culture of *Macrophomina phaseolina*

The isolate of *Macrophomina phaseolina* produced dark black to brown, raised colony on potato dextrose agar media. Mycelium was well developed, hyaline and septate. The fungus produced numerous sclerotia on host and also in culture medium (PDA). The sclerotia were more or less round with an exception of oval to irregular in shape. The present finding corroborates with the morphological characters reported by Singh R.S. (1998) [11], such as black, smooth, hard round to oblong or irregular shape of sclerotia. They measure about 100 microns to 1 mm in diameter (in culture 50 to 300 microns). However size is highly variable within an isolate.

A total of fifty two genotypes of mungbean were screened during *Kharif*, 2016-17 against *Macrophomina* blight under field condition. The observations on per cent blight intensity were recorded and test entries of mungbean were graded and categorized as Resistant (0 %), moderately resistant (0.1 to 10 %), moderately susceptible (10.1 to 25 %), susceptible (25.1 to 50 %) and highly susceptible (> 50 % PDI).

According to the results, 52 entries have shown different reactions against the pathogen. Out of fifty two entries, twenty one genotypes i.e. KPM 16-4, KPM 16-8, KPM 16-11, KPM 16-14, KPM 16-19, KPM 16-20, KPM 16-23, KPM 16-26, KPM 16-28, KPM 16-29, KPM 16-31, KPM 16-35, KPM 16-37, KPM 16-38, KPM 16-40, KPM 16-41, KPM 16-46, KPM 16-47, KPM 16-49, KPM 16-51, KPM 16-52 were found resistant (0%). Sixteen genotypes i.e. KPM 16-1, KPM 16-3, KPM 16-6, KPM 16-9, KPM 16-10, KPM 16-13, KPM 16-15, KPM 16-17, KPM 16-30, KPM 16-32, KPM 16-33, KPM 16-34, KPM 16-39, KPM 16-44, KPM 16-45, KPM 16-48 were found moderately resistant (0.1-10%). Likewise, five genotypes i.e. KPM 16-5, KPM 16-25, KPM 16-27, KPM 16-36, KPM 16-42 were found moderately susceptible (10.1-25%). However, nine genotypes i.e. KPM 16-2, KPM 16-7, KPM 16-12, KPM 16-16, KPM 16-18, KPM 16- 21, KPM 16-22, KPM 16-24, KPM, KPM 16-43 were found susceptible (25.1-50%) and one genotype i.e. KPM 16-50 found highly susceptible (>50 %).

Table 2: Screening of mung bean entries for their reaction to *Macrophomina* blight

S. No.	Score	Reaction	Frequency distribution	Entries
1.	1	Resistant	21	KPM 16-4, KPM 16-8, KPM 16-11, KPM 16-14, KPM 16-19, KPM 16-20, KPM 16-23, KPM 16-26, KPM 16-28, KPM 16-29, KPM 16-31, KPM 16-35, KPM 16-37, KPM 16-38, KPM 16-40, KPM 16-41, KPM 16-46, KPM 16-47, KPM 16-49, KPM 16-51, KPM 16-52
2.	2	Moderately resistant	16	KPM 16-1, KPM 16-3, KPM 16-6, KPM 16-9, KPM 16-10, KPM 16-13, KPM 16-15, KPM 16-17, KPM 16-30, KPM 16-32, KPM 16-33, KPM 16-34, KPM 16-39, KPM 16-44, KPM 16-45, KPM 16-48
3.	3	Moderately susceptible	5	KPM 16-5, KPM 16-25, KPM 16-27, KPM 16-36, KPM 16-42
4.	4	Susceptible	9	KPM 16-2, KPM 16-7, KPM 16-12, KPM 16-16, KPM 16-18, KPM 16- 21, KPM 16-22, KPM 16-24, KPM, KPM 16-43
5.	5	Highly susceptible	1	KPM 16-50
Total entries – 52				

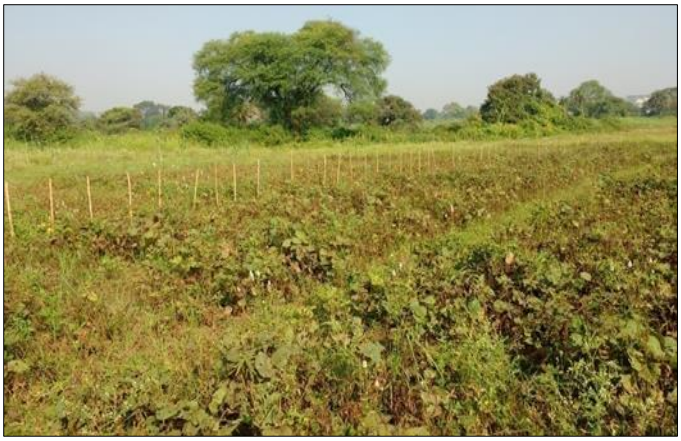

Plate 3: Field view of screening

Deepthi *et al* (2014) [3] revealed that only one entry PKDS-91 was found as moderately resistant to *Macrophomina* leaf blight of mungbean. Three entries (OSC-366-I, SSD-2-I and OSC-79) were recorded as moderately susceptible. Choudhary *et al* (2010) [2] evaluated 25 greengram entries in which complete resistance for *Macrophomina* leaf blight could not be found, however, 'MSJ 118' genotype exhibited highest suppression, followed by the genotype 'KM 4-59' and appeared as moderately resistant genotypes. Zote *et al.* (1983) [12] also found that out of 19 cultivars screened, none was completely free of *Macrophomina* blight; however 4 lines were moderately susceptible. Deshmukh (1991) observed resistant in BCG-1 out of 30 cultivars tested.

Conclusion

Out of fifty two entries, twenty one genotypes were found resistant. Sixteen genotypes were found moderately resistant. Likewise, five genotypes were found moderately susceptible. However, nine genotypes were found susceptible and one genotype found highly susceptible.

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