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Morphological and molecular characterization of *Macrophomina phaseolina* isolated from three legume crops and evaluation of mungbean genotypes for resistance to dry root rot

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ABSTRACT

Dry root rot (DRR) is an important emerging disease of mungbean caused by fungus *Macrophomina phaseolina*. The disease is seed and soil-borne and hence management is difficult. Exploitation of host resistance could be a good option to manage the disease. The objectives of this study were to characterize the isolates of *M. phaseolina* from three legume crops and to identify resistant sources against DRR of mungbean. Isolates of *M. phaseolina* from mungbean, urdbean, and vegetable soybean were identified using morphological characteristics and sequencing internal transcribed sequence (ITS) region of 18S rRNA. Isolates of *M. phaseolina* from urdbean and vegetable soybean were inoculated on susceptible check genotype of mungbean to assess their pathogenicity. Forty three mungbean genotypes were screened against *M. phaseolina* isolate of mungbean using the paper towel method to identify sources of resistance. Among these genotypes, 9 were resistant in repeated experiment, with disease score ranging from 1.9 to 3.0. Resistant genotypes showing lower disease score, namely IPM99-125, EC693368, and EC693369, were further screened using the sick pot method to confirm their resistance. Among these three genotypes, IPM99-125 showed consistently higher plant survival rate followed by EC693368 and EC693369 as compared to susceptible checks (VC3960-88, KPS1). In addition, these three genotypes were resistant when screened with two strains of *M. phaseolina* isolated from urdbean and vegetable soybean, with IPM99-125 having lower disease score. The mungbean genotype IPM99-125 could be useful in mungbean breeding programs to develop root rot-resistant varieties.

1. Introduction

Mungbean (*Vigna radiata* (L.) R. Wilczek var. *radiata*) is an economically important pulse crop in Asia and is a critical component of human diets as well as a major source of protein (Nair et al., 2012). Mungbean production has also been increasing in other parts of the world, including sub-Saharan Africa. Several fungal (Pandey et al., 2018) and viral diseases (Nair et al., 2017) pose challenges to mungbean production. Dry root rot (DRR) also called as charcoal rot is caused by *Macrophomina phaseolina* (Tassi.) Goid was reported as an emerging disease of mungbean several decades ago in South Asia, and the yield loss ranged from 25% to 48% (Bashir and Malik, 1988; Iqbal and Mukhtar, 2014). In recent years, the incidence of DRR has been increasing in Asia.

In mungbean, *M. phaseolina* causes substantial loss in the production by reducing plant population in the field, at both seedling and adult stages (Khan et al., 2016). In addition, the disease causes huge losses in the premium sprout market segment (Fuhlbohm et al., 2013). The pathogen is necrotroph and infects a wide range of crops. Roots of infected plants rot, plants wilt, and ultimately die when the disease reach at advance stages (Khan et al., 2017). In South and Southeast Asia, *Macrophomina* species causes diseases in diverse field crops, including common bean, (*Phaseolus vulgaris* L.), cowpea (*Vigna unguiculata* (L.), Walp), urdbean (*V. mungo* (L.) Hepper), soybean (*Glycine max* (L.) Merr.), potato (*Solanum tuberosum* L.), and cotton (*Gossypum hirusitum* L.) (Suriachandraselvan et al., 2005). During infection on host plants,

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the fungus produces several cell wall-degrading enzymes (Javaid and Saddique, 2011), hydrolytic enzymes (Kaur et al., 2012), and phytotoxins such as phaseolinone and botryodiplodin (Ramezani, 2008; Bressano et al., 2010).

Both morphological examination and molecular techniques were used to characterize isolates of *M. phaseolina* isolated from diverse legume crops (Babu et al., 2007; Sharma et al., 2012). Identification of *M. phaseolina* based on cultural and morphological features such as colony morphology, microscopic examination of microsclerotia, pycnidia, and conidia is not sufficient (Saleh et al., 2010). Biochemical and serological techniques are also used to identify the pathogen, but these techniques can identify only few species (Srivastava and Arora, 1997). Molecular methods such as RAPD analysis (Fuhlbohm et al., 2013), use of species-specific primers, LAMP (loop-mediated isothermal amplification)-based detection, sequencing of conserved gene, and internal transcribed spacers (ITS) of 18S rRNA (Ghosh et al., 2017) are commonly used to identify fungal pathogens (Babu et al., 2010). In addition, multilocus sequence analysis of housekeeping genes (such as calmodulin, histone H3, and translation elongation factor 1-alpha genes) is also being used to identify and characterize fungal plant pathogens (Joshi et al., 2006; Iqbal and Mukhtar, 2014).

The management of DRR of mungbean is challenging as the causal agent is a soil- and seed-borne pathogen. Chemical control of the soil-borne fungus is difficult and not economical for small holder farmers. The use of biocontrol agents and botanical extracts in combination with chemical fungicides provided good control of DRR of mungbean under controlled environments (Sundaramoorthy et al., 2013; Kumari et al., 2015; Javaid and Saddique, 2011). However, these biocontrol products are not commercially available and also require further evaluation in fields. If available, use of host resistance would be one of the best options to manage DRR of mungbean (Fuhlbohm et al., 2013). In recent years, researchers identified DRR resistant genotypes in mungbean (Khan and Shuaib, 2007; Choudhary et al., 2011). Additional resistant genotypes from diverse genetic resources are required to develop DRR resistant varieties. Therefore, the major goal of this study was to identify sources of DRR resistance of mungbean. Specific objectives were as follows: (i) to isolate and identify the causal agents of DRR from mungbean, urdbean, and vegetable soybean and use them in screening mungbean genotypes and (ii) to identify the sources of DRR resistance by screening 43 mungbean genotypes, which were selected based on their agronomic performance and other desirable traits in the previous studies (Sharma et al., 2016; Nair et al., 2017).

2. Materials and methods

2.1. Pathogen

DRR samples of mungbean, urdbean, and vegetable soybean infected with *M. phaseolina* were collected from the field of World Vegetable Center South Asia located in Hyderabad, India (N 17° 30.085′, E 078° 16.616′, Elevation: 550 m) in 2016. The samples were kept in pre-sterilized polyethylene bags separately and brought to the lab for further processing. Roots of diseased plants were cut in small pieces and surface sterilized with 70% ethanol or 2% Clorox (sodium hypochlorite) solution for 2 min and rinsed with sterile water. Pieces of surface disinfected diseased tissue were placed on potato dextrose agar (PDA, Himedia, India) plates and were incubated at 28 °C for 6–7 days. Culture of each isolate was purified by single sclerotial isolation, maintained on PDA slants and stored at 4 °C for further study. The fungal isolates were sent to National Center of Fungal Taxonomy, New Delhi, for identification based on morphological characteristics such as colony characteristics and morphology of microsclerotia and pycnidia characteristic etc. (Dhingra and Sinclair, 1978). For the long term storage, all the three fungal isolates were submitted to National Center of Fungal Taxonomy, New Delhi.

2.2. Molecular identification of the pathogen

The fungal isolates isolated from mungbean, urdbean, and vegetable soybean were identified by sequencing ITS1 and ITS2 regions of the 18S rRNA gene. The genomic DNA of each isolate was extracted by detergent (10% CTAB) and high salt (1.5 M)-based heat lysis (65 °C) method as described by Moller et al. (1992). The genomic DNA (gDNA) quantity was determined by measuring the absorbance at 260 nm using Thermo Scientific Nano Drop 1000 spectrophotometer (Thermo Scientific). The quality of extracted gDNA was accessed by subjecting them on 0.8% agarose gel electrophoresis and taking the absorbance ratio at 260:280 nm. The rDNA gene cluster consisting of ITS1, 5.8S rDNA and ITS2, was amplified using primer 5′-TCCGTAGGTGAACCTGCGG-3′ for ITS1 and 5′-GCTGCGTTCTTCATCGATGC-3′ for ITS 2 (White et al., 1990). Each PCR reaction mixture consisted of 5–10 ng of gDNA and 5 μM each of the primers ITS1 and ITS2. PCR reaction was performed using SUPERZym. Taq DNA Polymerase available from KPC Life Sciences. Reactions were performed in the provided buffer according to the manufacturer's protocol in a total volume of 50 μl. The reaction mixture was amplified in thermo cycler (BioRad) with the following amplifying conditions: initial denaturation at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 50.2 °C for 30 s, extension at 72 °C for 30 s, and final extension at 72 °C for 10 min. PCR-amplified products were fractionated on 2% agarose gel in 0.5X TAE buffer. ITS amplified bands of interest were purified from agarose gel using UniPro Gel Extraction Kit (KPC Life Sciences). Gel-purified DNA was used for sequencing reaction and sequenced by Macrogen Inc., Korea. Sequencing of the ITS portion from the fungal isolates was performed for both sense and antisense directions. Sequences were annotated and analyzed at BLAST to search the closest homolog. To confirm the sequence identity, BLAST search was conducted in the GenBank (National Center for Biotechnology Information, NCBI).

Table 1
Disease rating scale for DRR reaction[a].

Score	Description	Inferred Reaction type
1	No infection	Immune
>1 and ≤ 3	Very few small lesion on roots (1.1–2.0 = approximately 5% of root tissue covered with lesions, 2.1–3.0 = approximately 10% of root tissue covered with lesions)	Resistant
>3 and ≤ 5	Lesions on root clear but small, new root free from infection (3.1–4.0 = approximately 17.5% or the root tissue covered with lesions, 4.1–5.0 = approximately 25% of the root tissue covered with lesions)	Moderately resistance
>5 and ≤ 6	Lesions on roots are moderate, new roots generally free from infection (5.1–6.0 = approximately 37.5% of the root tissue covered with lesions)	Moderately susceptible
>6 and ≤ 8	Lesions on roots many, new roots generally free from infection (approximately 6.1–7.0 = 50% of the root tissue covered with lesions, 7.1–8.0 = approximately 62.5% of the root tissue covered with lesions)	Susceptible
>8 or 9	Roots infected and completely discolored (8.1–9.0 = approximately 75% infection)	Highly susceptible

[a] (Nene et al., 1981; Van Schoonhoven and Pastor-Corrales, 1987; Khan and Shuaib, 2007).

Table 2
Disease reaction of mungbean genotypes in the paper towel method.

Treatment	Trial 1		Trial 2	
Mungbean genotypes	Disease Score ± SD	Disease reaction category	Disease Score ± SD	Disease reaction category
EC693356 (VC6153B–20P)	6.3 ± 0.2	S	5.2 ± 0.4	MS
EC693357 (VC6465-8-5-2)	4.5 ± 0.7	MR	2.5 ± 0.8	R
EC693358 (VC6469-12-3-4A)	4.4 ± 0.09	MR	2.2 ± 0.1	R
EC693360 (VC6486-10-51)	7.5 ± 0.7	S	4.6 ± 0.08	MR
EC693361 (VC6489-9-1)	8.1 ± 0.6	HS	5.5 ± 0.7	MS
EC693362 (VC6492-59A)	6.2 ± 0.2	S	4.0 ± 0.08	MR
EC693363 (VC6493-44-1)	7.0 ± 0.8	S	5.3 ± 0.6	MS
EC693364 (VC6506-127)	2.6 ± 0.2	R	2.1 ± 0.6	R
EC693365 (VC6510-151-1)	7.1 ± 0.6	S	4.3 ± 0.1	MR
EC693366 (VC6512-6A)	2.1 ± 0.2	R	3.1 ± 0.4	MR
EC693367 (PDMA54)	7.1 ± 1.1	S	6.0 ± 0.9	MS
EC693368 (PUSA 9072)	2.0 ± 0.0	R	2.2 ± 0.0	R
EC693369 (TV03980A-G)	2.5 ± 0.5	R	2.2 ± 0.1	R
EC693370 (TV03717B-G)	5.6 ± 0.5	MS	5.6 ± 0.1	MS
EC693371 (TV01493A-G)	7.3 ± 0.5	S	4.4 ± 0.2	MR
EC693372 (VO1352B-G)	8.3 ± 0.5	HS	3.7 ± 1.0	MR
EC693374 (VO6381A-G)	6.0 ± 0.5	MS	2.7 ± 0.2	R
EC693376 (TV03719A-G)	5.6 ± 1.0	MS	5.5 ± 0.08	MS
HARSHA	5.2 ± 0.5	MS	5.3 ± 0.2	MS
IPM-02-14	5.3 ± 0.6	MS	4.5 ± 0.2	MS
IPM-02-17	2.3 ± 0.4	R	2.5 ± 0.3	R
IPM-02-3	2.2 ± 0.2	R	2.9 ± 0.5	R
IPM205-7	2.3 ± 0.3	R	2.4 ± 0.3	R
IPM99-125	2.0 ± 0.0	R	1.9 ± 0.4	R
KPS1	8.0 ± 0.0	S	6.1 ± 0.4	S
KPS2	6.0 ± 0.0	S	5.4 ± 0.2	MS
ML1299	7.4 ± 0.04	S	4.7 ± 0.2	MR
ML1628	6.0 ± 1.3	S	6.3 ± 0.2	S
ML1666	5.6 ± 0.1	MS	5.6 ± 0.1	MS
ML818	3.7 ± 0.6	MR	3.8 ± 0.5	MR
NM94	6.0 ± 0.7	MS	4.2 ± 0.5	MR
NM92	5.5 ± 0.0	MS	5.6 ± 0.08	MS
PAU911	6.1 ± 0.6	S	6.7 ± 0.5	S
PDM139	3.0 ± 0.3	R	3.7 ± 0.5	MR
VC 3960-88	8.3 ± 0.4	HS	7.7 ± 0.3	S
VC 6153 B-20	5.7 ± 1.7	MS	5.6 ± 0.08	MS
V04718	2.8 ± 0.4	R	2.8 ± 0.5	R
VC 6368 (46-40-4)	4.1 ± 0.6	MR	5.0 ± 0.9	MS
VC 6369 (53–97)	5.3 ± 0.2	MS	5.3 ± 0.1	MS
VC3890	5.2 ± 0.5	MS	3.9 ± 0.3	MR
VC6173 B-10	2.5 ± 0.1	R	2.2 ± 0.0	R
VC6368 (46-40-1)	5.3 ± 0.3	MS	5.1 ± 0.1	MS
VC6372 (45-8-1)	5.3 ± 0.2	MS	5.6 ± 0.1	MS

DRR disease score was rated on 1–9 rating scale (Nene et al., 1981; Van Schoonhoven and Pastor-Corrales, 1987, Khan and Shuaib, 2007) where 1 = Immune and 9 = Highly susceptible, Chi square = 123.4861 (trial 1), 121.0769 (trial 2), DF = 44, $p < 0.0001$, SD = Standard deviation, R = Resistant, MR. = Moderately resistant, MS = Moderately susceptible, S=Susceptible, HS=Highly susceptible.

2.3. Screening of mungbean genotypes for DRR

Forty three mungbean genotypes were first evaluated (Table 2) for DRR using paper towel method (Khan and Shuaib, 2007). Mungbean genotypes with lower disease score were further evaluated for DRR using the sick pot method as described in Choudhary et al. (2011) in the glasshouse. Experiments were conducted in 2016 and 2017 at the World Vegetable Center South Asia, Hyderabad (India).

2.3.1. Plant materials

Mungbean seeds of 43 genotypes available at the World Vegetable Center South Asia, Hyderabad, were planted in a pro tray containing sterilized soil mixture (autoclaved black sandy soil) and kept in the glasshouse at 25 °C for eight days to raise seedlings.

2.3.2. Experimental design

Two independent trials were conducted for all experiments described in the following paragraphs. Each experiment had three replicates and arranged in a completely randomized design (CRD) for paper towel experiments and randomized completely block design (RCBD) for sick pot experiment.

2.3.3. Screening of mungbean gentoypes against mungbean isolate of *M. phaseolina*

2.3.3.1. Screening of mungbean genotypes by the paper towel method. The inoculum of *M. phaseolina* isolated from mungbean was prepared by inoculating mycelial disc (6 mm diam) of the pathogen in conical flasks (250 ml) having potato dextrose broth (PDB, Himedia, India) for 10 day at 28 °C in a BOD (Biological Oxygen Demand) incubator (Thermo Fishers Scientific Inc., Germany). After ten days of incubation, fungal mycelia mat from each flask was filtered, weighed (16 g) and ground in a blender by mixing with 50 ml of sterile water. The prepared mycelial suspension was kept in a sterilized screw cap vial at 4 °C for further experiments. Eight days old seedlings of mungbean were uprooted and roots were washed with running tap water, then rinsed with sterilized water. Uprooted seedlings of individual genotypes were kept in pre-sterilized polyethylene bags separately and were brought to the laboratory for further processing. Seedlings of each genotype were made into three bunches for three replicates in the experiment. Each bunch had 10 seedlings. Mycelia mat from one flask (50 ml) was used for the inoculation of 9 bunches of seedlings. Roots of seedlings were dipped into the fungal suspension for ~60 s and thereafter seedlings were placed side by side on a sterilized paper towel so that only leaves and one cm of stem remained outside of the paper towel. Control seedlings were dipped in sterile water and kept in paper towel as described above. The prepared paper towels were labelled with individual genotypes. After arranging the paper towels in lots of 10 in a tray according a completely randomized design (CRD), the seedlings were kept inside an incubator at a temperature of 35 °C and with a photoperiod of 12 h for the disease development. The moisture in seedlings with a paper towel was maintained by sprinkling water daily (Sharma and Pande, 2013). Two independent trials were carried out with three replicates. Disease severity was recorded after seven days using rating scale 1–9 as described in Table 1 (Nene et al., 1981; Van Schoonhoven and Pastor-Corrales, 1987; Khan and Shuaib, 2007).

2.3.3.2. Screening of resistant mungbean genotypes by sick pot method. The isolate of *M. phaseolina* isolated from mungbean was multiplied on sorghum grains using methods as described by Choudhary et al. (2011). The inoculum grown in sorghum was ground in a blender and a 50 g of the inoculum was mixed with 1 kg of sterilized soil and the soil mixture was incubated for 1 week at room temperature for the colonization of the fungus. Two kg of the inoculated soil mixture was transferred to 6 inches diameter plastic pots. The soil moisture in pots was maintained

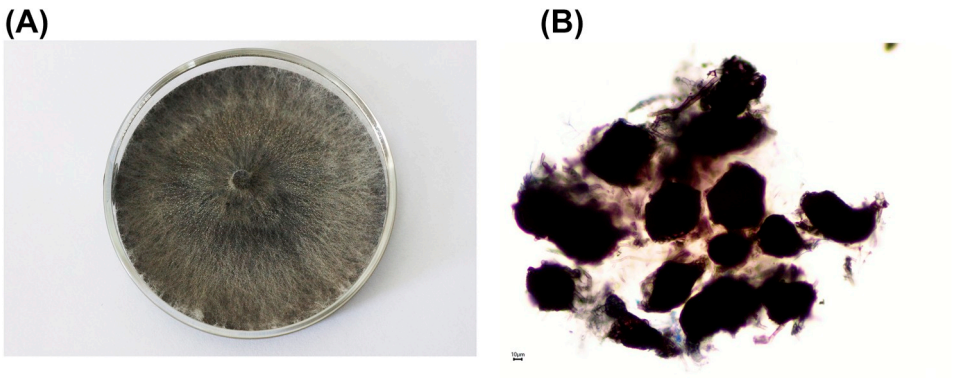

Fig. 1. Cultural and morphological characteristics of *M. phaseolina* a: Colony on the agar plate, b: microsclerotia.

by watering pots as required (~60% soil moisture). After 5 days, before commencement of the experiment, 10 seeds of susceptible genotype (VC3960-88) were sown in sick pots and pots were kept in glasshouse conditions (~32 ± 2 °C) to check whether the inoculated pathogen was pathogenic or not. Once mortality of the susceptible genotypes reached >90%, these pots were used for the screening of resistance genotypes obtained through paper towel method. Ten seeds of each resistant genotypes (EC693368, IPM99-125, EC693369) and susceptible checks (KPS1, VC3960-88) were planted in individual pots. The pots were arranged in a randomized complete block design (RCBD) in three replications in the glasshouse conditions. The susceptible checks grown in pathogen free soil were used as control. Percent survival of the each genotype was recorded once mortality in susceptible checks reached >90% (45–55 days after sowing). Percent survival rate was calculated as 100 × Number of survived plants/total number of plants (Pawlowski et al., 2015).

2.4. Screening of mungbean resistant genotypes against M. phaseolina isolates isolated from urdbean and vegetable soybean

Pathogenicity test of *M. phaseolina* isolates from urdbean and vegetable soybean was conducted on the mungbean susceptible genotype, VC3960-88, using the paper towel method. To verify Koch's postulate, *M. phaseolina* was re-isolated from the representative inoculated plants with DRR symptoms. Disease severity was recorded seven days after inoculation using 1–9 rating scale (Nene et al., 1981; Khan and Shuaib, 2007; Van Schoonhoven and Pastor-Corrales, 1987; Table 1). Once pathogenicity was confirmed on mungbean genotype VC3960-88, these two isolates (*M. phaseolina* from urdbean and soybean) were used for the screening of three resistant mungbean genotypes using paper towel method. Seedlings of resistant (EC693368, IPM99-125, EC693369) and susceptible (KPS1, VC3960-88) genotypes were grown in the glasshouse. Fungal inoculum from isolates of *M. phaseolina* isolated from urdbean and vegetable soybean was prepared as described earlier. Eight-day-old seedlings' roots were dipped into the fungal inoculum for about 60 s, and the seedlings were then placed side by side on a paper towel. As described earlier, seedlings were wrapped in paper towels and were kept inside an incubator (35 °C) with a photoperiod of 12 h after arranging them in complete randomized design (CRD). Disease severity of infected seedlings was recorded at seven days after inoculation using 1–9 rating scale (Table 1).

2.5. Data analysis

Combined and trial-wise analysis of variance was performed to test the significance of trial, genotypes and interaction of trial and genotypes effect (trial x genotypes) using MIXED procedure of SAS (SAS Institute Inc. 2015). Individual trial residual variances were modeled into combined analysis using repeated statement in MIXED procedure. BLUEs (Best Liner Unbiased Estimators) for trial x genotypes were estimated from combined analysis of variance. Square root transformation applied for disease score and number of plants wilted traits, BLUEs were retransformed with square transformation.

3. Results

3.1. Morphological and molecular characterization of Macrophomina phaseolina

Isolates of *Macrophomina phaseolina* isolated from mungbean,

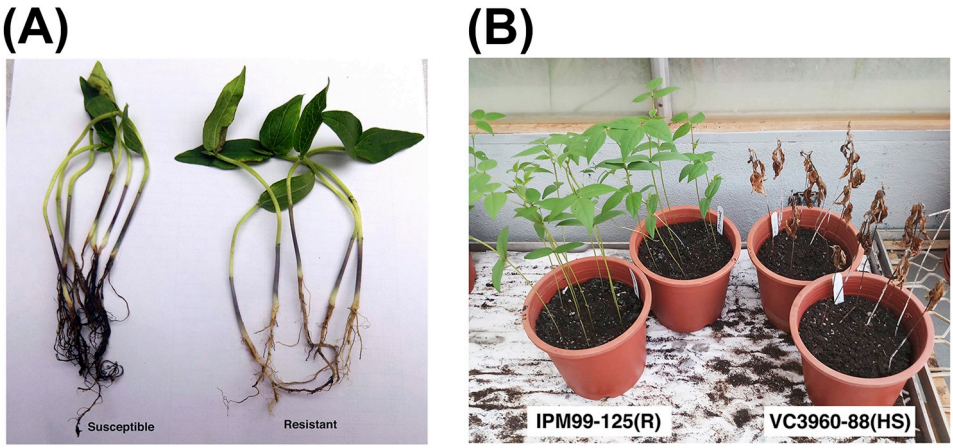

Fig. 2. Mungbean genotypes showing symptoms of DRR and disease reaction in paper towel (a) and sick pot methods (b).

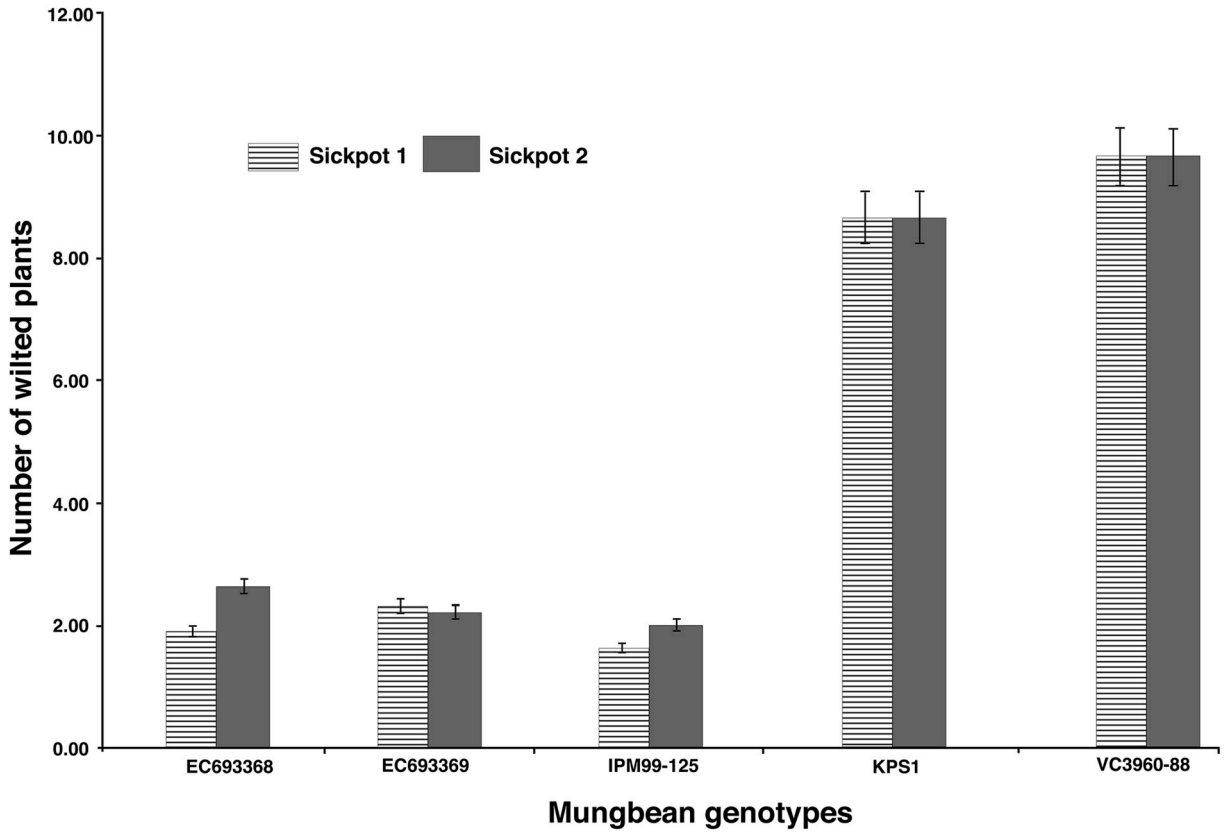

Fig. 3. Interaction plot to study the mean performance of wilted plants in identified resistant and susceptible genotypes of mungbean in sick pot trial 1 and trial 2 conducted in glasshouse.

Table 3
Disease reaction of identified resistant mungbean genotypes against *M. phaseolina* isolated from urdbean and vegetable soybean.

Mungbean genotypes	Disease score ± SD		Disease reaction category
	MPU	MPS	
EC693368	2.4 ± 0.2^{c}	2.4 ± 0.3^{c}	R
EC693369	2.3 ± 0.1^{c}	2.0 ± 0.09^{dc}	R
KPS1	7.2 ± 0.1^{b}	7.1 ± 0.5^{b}	S
IPM99-125	1.7 ± 0.1^{d}	1.9 ± 0.2^{d}	R
VC3960-88	8.1 ± 0.3^{a}	7.8 ± 0.5^{a}	MPU:HS, MPS:S

DRR disease score was rated on 1−9 rating scale where 1= Immune and 9= Highly susceptible, MPU: *Macrophomina phaseolina* from urdbean, MPS: *M. phaseolina* from vegetable soybean, within individual row values with different superscript are significantly different from each other at $p<0.0001$, SD = Standard deviation, MPU: CV=3.7, R-square=0.99, DF=4, F−value=554.81; MPS: CV=5.1, R-square=0.98, DF=4, F−value=293.85. R=Resistant, S= Susceptible, HS= Highly Susceptible.

urdbean, and vegetable soybean were characterized using morphological characteristics and sequencing portion of ITS. Morphological characteristics of all three isolates were similar to typical characteristics of *M. phaseolina*. Colonies were dark brown-greyish in color initially whitish on the PDA medium (Fig. 1a). Semi-appressed mycelium was observed on the culture plate with microsclerotia imbedded within the hyphae or engrossed in the agar. Aggregation of hyphae formed jet black color microsclerotia with 100–120 μm in size (Fig. 1b). Microsclerotia were smooth and irregular in shape, and some were round to oblong. *M. phaseolina* isolates isolated from mungbean and vegetable soybean showed dense growth, while that isolated from urdbean showed feathery growth. Sequence of ITS portion of 18S rRNA of all the three isolates isolated from mungbean, urdbean, and vegetable soybean were identical. These sequences showed 99% similarity with the ITS sequences of *M. phaseolina* isolates from common bean, (KU831500.1), cowpea, (KF951783.1), mungbean (KF951636.1), urdbean (KF951637.1), potato (KU721993.1), and cotton (KX270356.1) in BLAST search. Because ITS sequences of all three isolates of *M. phaseolina* were identical, sequence of one isolate (mungbean) was deposited to gene bank (accession no: MN006689).

3.2. Screening of mungbean genotypes against M. phaseolina isolate of mungbean by the paper towel method

Evaluation of 43 mungbean genotypes against *M. phaseolina* isolate of mungbean through the paper towel method revealed considerable variation in their resistance levels (Table 2). DRR symptoms of resistant and susceptible genotypes are shown in Fig. 2a. The Chi-square test showed a significant ($p > 0.0001$) difference between trials 1 and 2. In trial 1, 11 and 4 genotypes were resistant and moderately resistant, respectively, and 13, 12, and 3 genotypes were moderately susceptible, susceptible, and highly susceptible, respectively (Table 2). In trial 2, 12 and 11 genotypes were resistant and moderately resistant and 16 and 4 genotypes were moderately susceptible and susceptible, respectively. Nine genotypes showed consistently resistant reaction in both trials.

3.3. Screening of resistant mungbean genotypes by the sick pot method

Three mungbean genotypes (IPM99-125, EC693368, and EC693369), which showed consistent resistant reactions and lower disease score in both trials in the paper towel assay, were further evaluated in the sick pot assay. In this experiment, the variances of the repeated trials (sick pot 1 and sick pot 2) were not significantly different (Fig. 3), and the data were combined for analysis. There were significant differences among resistant and susceptible mungbean genotypes for percentage plant survival ($p < 0.0001$). Genotypes IPM99-125 (81.9%), EC693368, and EC693369 (77.4%) showed significantly higher percentage of plant survival (CV = 10.11300, r-square = 0.96, DF = 4, F

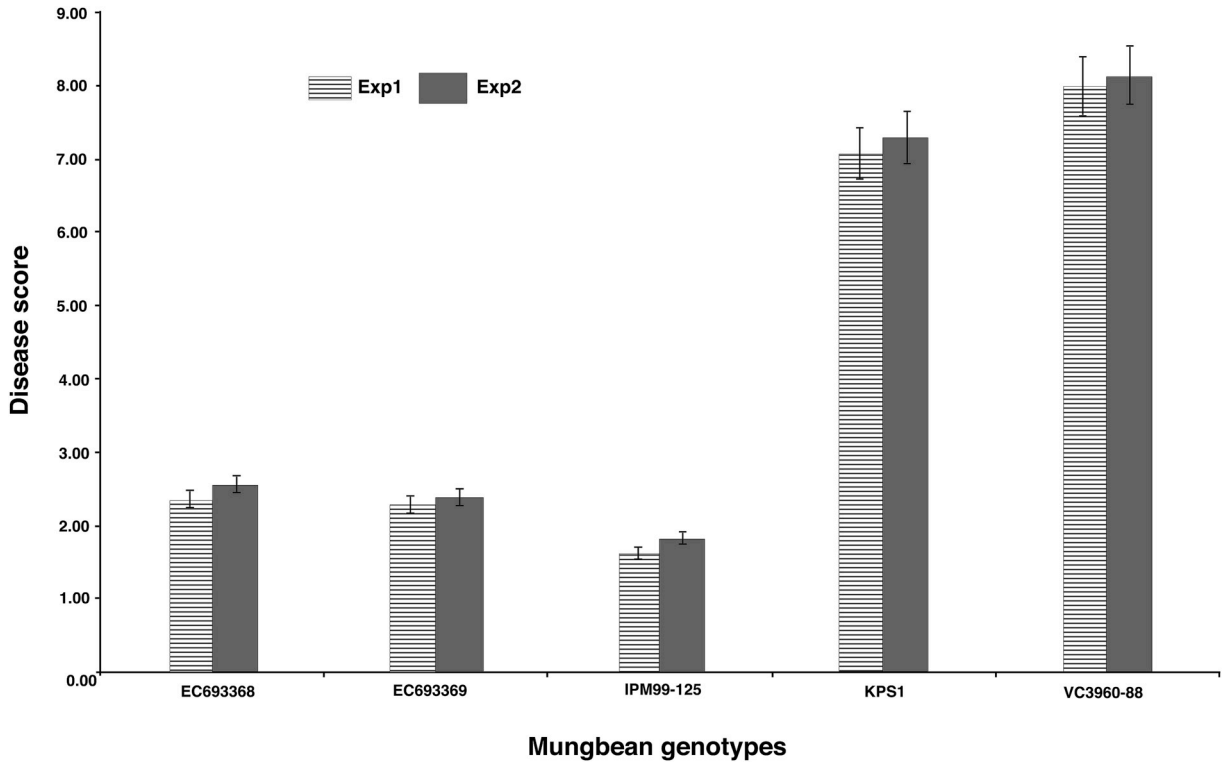

Fig. 4a. Interaction plot to study the mean performance of dry root rot reaction in identified resistant and susceptible genotypes of mungbean in paper towel experimental trials 1 and 2 against *M. phaseolina* from urdbean.

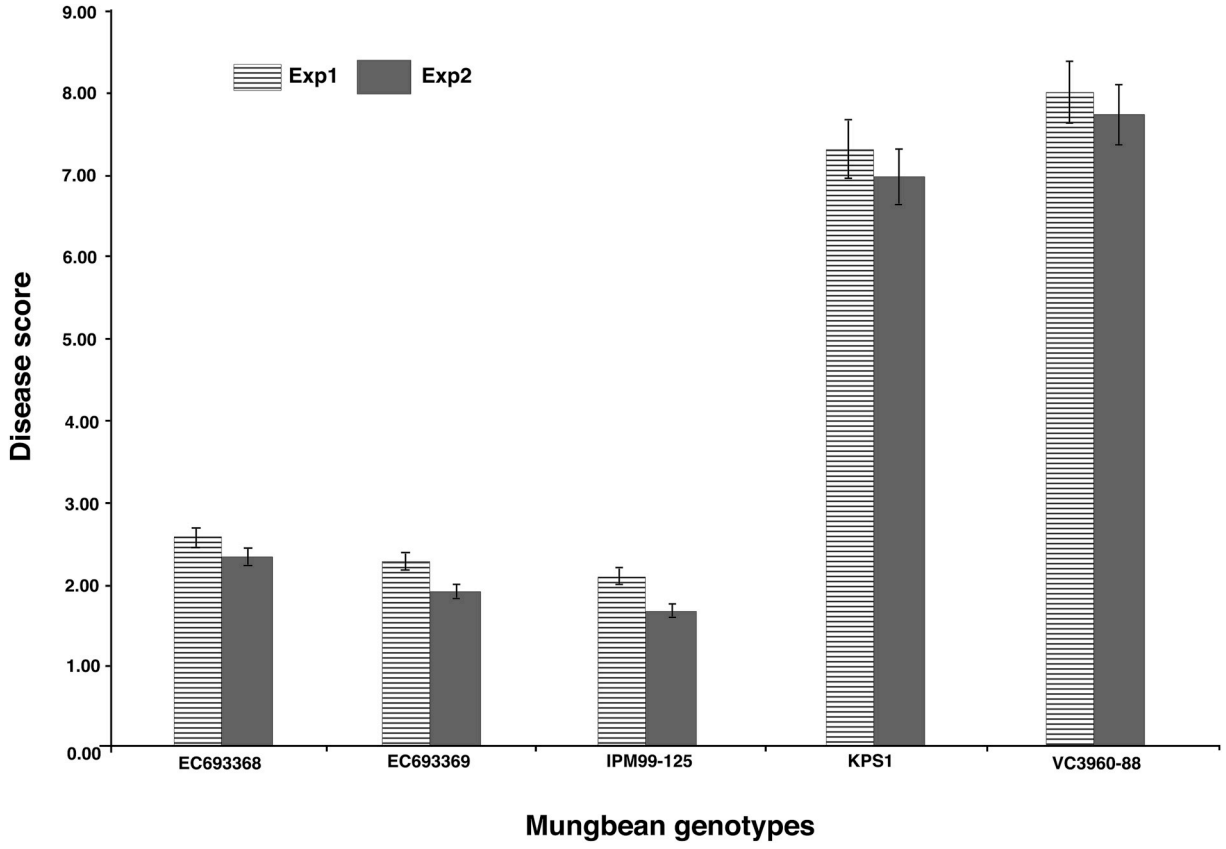

Fig. 4b. Interaction plot to study the performance of dry root rot reaction in identified resistant and susceptible genotypes of mungbean in paper towel experimental trials 1 and 2 against *M. phaseolina* from vegetable soybean.

values = 101.78) than the susceptible checks VC3960-88 (3.4%) and KPS1 (13.4%) (Fig. 2b). The error variance was homogeneous across the groups.

3.4. Screening of mungbean resistant genotypes against M. phaseolina isolates isolated from urdbean and vegetable soybean

Evaluation of three resistant genotypes (IPM99-125, EC693368, and EC693369) conducted through the paper towel assay by inoculating with *M. phaseolina* isolates isolated from urdbean and vegetable soybean showed that these genotypes were resistant (Table 3). The disease score of the repeated experiments (Exp 1 and Exp 2) were not significantly different (Fig. 4a and b), and the data of experiments 1 and 2 were combined for analysis. Disease scores of resistant genotypes IPM99-125, EC693368, and EC693369 were significantly ($p < 0.0001$) lower than those of susceptible genotypes. The error variance was homogeneous across the groups.

4. Discussion

Macrophomina is a polyphagous pathogen causes DRR or charcoal rot disease in several economically important crops such as legumes and vegetables (Kaur et al., 2012; Kumar et al., 2017). In vegetable legumes, mungbean, urdbean, and vegetable soybean are major hosts of *M. phaseolina.* In this study, *M. phaseolina* isolated from three commonly grown legume crops (mungbean, urdbean, and vegetable soybean) have been characterized through morphological characteristics and ITS sequencing. There were some cultural variations among the isolates; however, morphological characteristics of the three isolates were similar to each other. ITS sequences of these three isolates were identical and BLAST search in the GenBank confirmed *M. phaseolina.* Several previous studies also used ITS sequencing of 18S rRNA region to identify *M. phaseolina* from different hosts including mungbean (Babu et al., 2007; Romanelli et al., 2014; Khan et al., 2017).

The management of DRR of mungbean and other legume crops is challenging, as the fungus is seed- and soil-borne and can survive in soil for several years. In developing countries, the use of fungicides such as carbendazim is not economical and reliable to control dry root rot at the grower's level. In this context, growing disease resistant varieties would be the best option to manage DRR of mungbean, if available. For this purpose, the identification of sources of resistance is the first step. In this study, 43 mungbean genotypes from diverse sources were evaluated using the paper towel assay in controlled environments in two independent trials. Among these genotypes, nine of them showed consistent resistant reactions in both the trials. These resistant genotypes could be used in disease resistance programs after proper evaluation in the fields. Ten genotypes that showed resistance or moderately resistance reaction in trial 2 were showed susceptible or moderately susceptible reactions in trial 1. The phenotypic variation between two trials could be due to different responses of genotypes to the environments in two trials or quantitative nature of disease resistance.

The paper towel screening assay was first used to evaluate all 43 genotypes. Among these genotypes, three genotypes (IPM99-125, EC693368, and EC693369) having consistent resistant reactions were further evaluated using the sick pot method. For the rapid identification of the resistant genotype in mungbean against DRR, the paper towel assay was very useful (Sharma et al., 2015) as it saved resources, time, and space. One limitation of this method is that the seedlings are exposed to the pathogen for only a limited period. Therefore, it is always better to evaluate the selected genotypes in the sick pot assay (60–80 days) or in field, as these methods provide longer period for host-pathogen interactions. Higher survival of plants of IDM99-125 genotype than susceptible checks in the sick pot method indicated that this genotype may be useful as parents for developing mungbean cultivars with DRR resistance. The three resistant genotypes (IPM99-125, EC693368, and EC693369) identified through the paper towel method also showed resistance against *M. phaseolina* isolates isolated from urdbean and vegetable soybean. Thus, these genotypes could be useful to protect mungbean and other closely related legume crops from *M. phaseolina* causing DRR. With these resistant genotypes, farmer can perform crop rotation of mungbean with urdbean and soybean, where DRR is the major problem.

Other researchers also used the paper towel method for the screening of mungbean genotypes against DRR. Khan (2008) screened 29 varieties of mungbean against DRR by the paper towel method and found that two varieties, namely NCM 252–10 and 40536, were highly resistant; however, in the present study, none of the genotypes showed highly resistant reaction. This may be due to variation in aggressiveness of *Macrophomina* species occurring at different eco-climatic regions (Kumar et al., 2017) and due to different genetic materials utilized for the screening. The usefulness of the sick pot/sick plot method to determine the sources of resistance in mungbean against DRR has been reported only by a few researchers. Among 26 mungbean genotypes were screened from Pakistan by the sick pot method, 2 genotypes (MNUYT-317 and NM-2011) showed highly resistant reaction with high survival of the plants (Khan et al., 2016). Few researchers reported that at the field level, mungbean genotypes MSJ 118, KM 4–44, and KM 4–59 (Choudhary et al., 2011, India), and Azri 2006, NM 2006, and AUM 9 (Haseeb et al., 2013, Pakistan) showed resistance to DRR. In the present study, 9 mungbean genotypes showed consistent resistant reactions against DRR in the paper towel method. Out of these 9 mungbean genotypes, IPM99-125, EC693368, and EC693369 having lower disease score also showed higher plant survival when tested by the sick pot method. Highest suppression of DRR in the mungbean genotypes viz. IPM-99125, EC693368 and EC693369 indicates proper root development, which in turn draws sufficient amount of moisture from soil, making water potential of the plants in the range that allows for minimum infection of *M. phaseolina*. The mungbean genotypes IPM99-125 and EC693369 that showed resistance against DRR in this study were also reported for other desirable traits such as MYMD (Mungbean Yellow Mosaic Disease) resistance (Nair et al., 2017) and heat tolerance (Sharma et al., 2016), respectively.

In mungbean, although there are reports on the resistant genotypes against DRR (Iqbal and Mukhtar, 2014; Gahlot, 2018), there are no reports on the nature of resistance. QTLs for *M. phaseolina* resistance was identified in sorghum (Reddy et al., 2008), beans (Hernández-Delgadoet al., 2009) and cowpea (Muchero et al., 2011). It would be important to understand the genetics of the resistance trait in the genotypes identified in the present study. In addition, mapping populations developed from the resistant sources would be required for mapping of the gene(s) for enabling marker-assisted selection.

5. Conclusion

The present study revealed 18S rRNA based identification of *M. phaseolina* isolated from mungbean, urdbean, and vegetable soybean. Paper towel and sick pot screening methods revealed the absence of complete resistance (0% disease incidence) to *M. phaseolina* in the present set of mungbean genotypes. However, cultivation of resistant elite genotype IPM99-125 could be recommended for mungbean production in disease problematic areas after multi-location field trials. Host resistance may be the best alternative for cost-effective and ecofriendly management of the disease. The resistant genotypes identified in the present studies could also be utilized as resistant donors for developing resistant varieties after successful field trials.

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