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Identification of new sources of resistance to dry root rot caused by *Macrophomina phaseolina* isolates from India and Myanmar in a mungbean mini-core collection

Abhay K. Pandey [a,b,*], Myint Yee [c], Mar Mar Win [c], Hnin Moh Moh Lwin [c], Gopikrishna Adapala [d], Abhishek Rathore [d], Zong-ming Sheu [e], Ramakrishnan M. Nair [a,**]

[a] World Vegetable Center, South Asia, ICRISAT Campus, Patancheru, Hyderabad, 502324, TS, India
[b] Tea Research Association, North Bengal Regional R & D Center, Nagrakata, 735225, West Bengal, India
[c] Department of Agricultural Research, Yezin, 15013, Myanmar
[d] International Crop Research Institute for the Semi-Arid Tropics, Hyderabad, 502324, TS, India
[e] World Vegetable Center, P.O. Box 42, Shanhua, Tainan, 74199, Taiwan

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ABSTRACT

Dry root rot (DRR), caused by *Macrophomina phaseolina*, is a prevalent disease of mungbean in Myanmar, and an emerging problem in South Asia. The pathogen is a polyphagous necrotroph, survives in the soil for many years that results disease mitigation difficult. Managing DRR in mungbean through an integrated approach has been suggested, and the use of resistant varieties is one of the economical methods. The present study aimed to identify sources of resistance against DRR from a mungbean mini-core collection and to characterize the associated *M. phaseolina* isolates from India and Myanmar. Evaluation of the 296 mungbean mini-core accessions against the isolate MP1 by paper towel method identified 29 accessions with DRR resistance (disease scores: ≤ 3), and 18 of them with the consistent resistance in the repeated experiment. During the screening of 18 resistant accessions in the glasshouse, nine accessions were found DRR resistance in repeated sick pot experiments with $\leq$10% disease incidence. A subset of 30 accessions was selected from the mini-core collection based on their *in vitro* DRR reactions. These accessions were evaluated for DRR resistance in the field in Yezin, Myanmar in 2018 and 2019. Out of the 30 accessions, ten accessions were found DRR resistance with $\leq$10% disease incidence in both years of evaluations. Pooled analysis of percent disease incidence data of 15 accessions common in both glasshouse and field revealed the stability of accessions VI001509AG, VI001244AG, and VI001400AG for DRR resistance across years and locations. The three resistant accessions along with a susceptible check VC693088 were re-evaluated by paper towel method against nine additional *M. phaseolina* isolates from India (MP3-MP11). The accessions VI001509AG and VI001400AG were resistant to all nine isolates, while accession VI001244AG was resistant to MP5, MP6, and MP7 isolates. These accessions could be used in mungbean DRR resistance breeding programs.

1. Introduction

The fungus *Macrophomina phaseolina* (Tassi) Goid, belongs to the class Botryosphaeriaceae order Botryosphaeriales, it is a necrotrophic pathogen and has a wide host range including mungbean (*Vigna radiata* (L.) R. Wilczek var. *radiata*). Worldwide, the pathogen infects more than 500 economically important crops. The pathogen is widespread on other hosts, such as cotton (*Gossypium hirsutum* L.), groundnut (*Arachis hypogaea* L.), chickpea (*Cicer arientinum* L.), urdbean (*Vigna mungo* L. (Hepper), soybean (*Glycine* max L.), and maize (*Zea mays* L.) (Sobti and Sharma, 1992; Aly et al., 2007; Bressano et al., 2010). Globally, mungbean is an important legume crop. It is a good source of proteins and rich in other essential nutrients (Nair et al., 2012). The crop is mostly grown in East, South, and Southeast Asia and in East Africa, and recently, it has expanded to Australia, sub-Saharan Africa, and South America (Mbeyagala et al., 2017; Nair et al., 2019; Noble et al., 2019).

* Corresponding author. World Vegetable Center, South Asia, ICRISAT Campus, Patancheru, Hyderabad, 502324, TS, India.
** Corresponding author. World Vegetable Center, South Asia, ICRISAT Campus, Patancheru, Hyderabad, 502324, TS, India.
E-mail addresses: abhaykumarpandey.ku@gmail.com (A.K. Pandey), ramakrishnan.nair@worldveg.org (R.M. Nair).

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Worldwide, the total cropped area of mungbean is about 7 million ha, with a total production of 5 million tons (Nair et al., 2019).

In South Asia, the mungbean production is constrained by many yield-limiting abiotic and biotic stresses (Nair et al., 2019). *Macrophomina phaseolina* (MP) causes dry root rot (DRR), which is a prevalent disease of mungbean in Myanmar, and an emerging problem in India and Pakistan (Bhatia and Raghavan, 2016; Pandey et al., 2020). The pathogen survives in soil for many years and is capable to infect mungbean plants at all phases of growth. A disease caused by this pathogen is more severe under higher temperatures and low soil moisture (Saleh et al., 2010). The pathogen spreads from one plant to other adjacent plants through the soil. It spreads through the vascular system within the infected plants (Gangopadhyay et al., 1970). The rotting of infected roots results in wilting in advanced stages and ultimately causes the death of plants (Choudhary et al., 2011). During infection, MP produces toxins such as botryodiplodin and phaseolinone, and these toxins help the pathogen to infect susceptible plants from soil reservoirs, particularly over the winter (Abbas et al., 2019). In recent years, DRR incidence has been spreading in Asia due to enhanced water stress during the crop period because of irregular and reduced rainfall and increase temperature. DRR has become a key emerging yield-limiting disease of mungbean in South Asian Countries (Singh et al., 2020). The management of DRR is challenging due to the continued survival of MP as microsclerotia in the soil for several years (Iqbal and Mukhtar, 2014).

Agricultural practices, such as timely sowing, application of fungicides and microbial bio-control agents as seeds' treatments, have been used for DRR management in mungbean (Pandey et al., 2018; Satya et al., 2011). Besides, plant extracts of *Carum copticum* and *Azadirachta indica* have shown efficacy against DRR (Iqbal et al., 2014). Nevertheless, fungicide applications are neither economical nor biodegradable, and bio-pesticides are not locally available to the growers. The use of resistant varieties is an alternative strategy because it is always efficient, compatible with its efficiency and compatibility with other management practices, and also it does not increase production costs. Although, the extensive efforts have been made to mitigate the DRR by screening mungbean accessions for DRR resistance (Khan and Shuaib, 2007; Khan, 2008; Pandey et al., 2020), the existing resistant accessions are still not sufficient to use against DRR in the field due to the high pathogenic variability of MP. Hence, new resistant accessions from mixed genetic resources are a prerequisite for the development of resistant varieties against DRR, as the virulence of pathogens might be altered due to climate change (Kumari and Ghatak, 2018).

Therefore, the objectives of the present study were: (i) to identify sources of resistance against DRR from a mungbean mini-core collection, and (ii) to characterize and compare the eleven isolates of MP from DRR symptomatic mungbean roots grown in different agro-climatic regions. We screened the mini-core collection to discover the newer sources of resistance to DRR, its variation for resistance to DRR, and their subsequent use in future resistance breeding programs. The isolates MP1 and MP2 were relatively more pathogenic and used for the screening of mini-core accessions. The identity of these isolates as MP was confirmed by sequencing the ITS region.

2. Materials and methods

2.1. Sample collection and isolation of the pathogen

In 2017, DRR symptomatic plants were uprooted and collected from a mungbean field of the World Vegetable Center, South Asia, Hyderabad (N 17° 30.085′, E 078° 16.616′, Elevation: 550 m), India in sterilized polyolefin bags. The samples were brought to the laboratory for pathogen isolation and identification. The associated pathogen was isolated from symptomatic roots on PDA (Potato Dextrose Agar, Himedia, India) plates as per standard mycological procedures (Sain and Pandey, 2018). A single isolate obtained from this location was designated as MP1. The pathogen was purified through a single sclerotia isolation method and preserved at 4 °C on agar slants.

Likewise, a total of ten additional isolates of MP were isolated from individual DRR infected root collected from mungbean field of Yezin, Myanmar (MP2), and major mungbean-growing states of India, namely Odisha (Odisha Agriculture University Campus, Bhubaneswar, MP3), Punjab (Ludhiana, MP4), Himachal Pradesh (CSK Agriculture University Campus, Palampur, MP5), Rajasthan (Mongrakalan, MP6; Jodhpur Agric. University Campus, MP7, Bijwaria, MP8, and Rampur, MP9), and Maharashtra (Masa, MP10, and Amravati, MP11). Each isolate of MP was preserved separately on agar slants at 4 °C.

2.2. Cultural and morphological variability among the MP isolates

Cultural and morphological variability among the eleven MP isolates were studied in the laboratory (Table 1). The parameters used to describe the cultural and morphological characteristics were, colony color, growth pattern, the formation of a septum in the branch near the origin, the pattern of sclerotia production, shape, size, and the number of microsclerotia (Dhingra and Sinclair, 1978). A 7-day-old culture of each isolate cultured on the PDA plate was used for examination of sclerotia morphology. The numbers of sclerotia were counted and recorded per 9 mm mycelial disc of each isolate in three replicates (Shekhar et al., 2006). The shape and size (average of 10 sclerotia) of microsclerotia of each isolate were examined separately, under a camera-attached-stereomicroscope (Dhingra and Sinclair, 1978). Each isolate (except MP2) was submitted to the National Center of Fungal Taxonomy, New Delhi, for confirmation at the species levels.

2.3. Pathogenicity tests

The pathogenicity test of each MP isolate from India was conducted on a mungbean susceptible genotype (VC3960-88) using the paper towel method (Nene et al., 1981). Precisely, the fungal inoculum was prepared from a 7-day-old culture of each MP isolate cultured separately in 100 ml Potato Dextrose Broth (Himedia, India) in conical flasks (250 ml). The mycelial mat (16 g) of each isolate was ground in a blender with sterile water to make the suspension. The mycelial suspension (50 ml) from one conical flask was used for the inoculation of nine bunches (each bunch contained ten seedlings) of seedlings.

In a glasshouse, to raise the seedlings, seeds of the mungbean genotype VC3960-88 were grown in plastic pro trays containing sterilized black-sandy soil mixtures. At 8 days, seedlings were uprooted and their roots were washed with sterilized water. The washed roots were dipped in the mycelial suspension (~60 s), and subsequently, seedlings were kept side by side on a pre-sterilized paper towel so that only 1–2 cm of the stem with leaves remained on the outside of the paper towel. For control sets, seedlings' roots were dipped in double-distilled sterilized water. The prepared paper towels were labelled with each MP isolate. The labelled paper towels with seedlings were arranged in a completely randomized design (CRD) in lots of 10 in a tray with three replications. The trays were kept at 35 °C and with a photoperiod of 12 h inside a BOD (Biochemical Oxygen Demand) incubator (Thermo Fishers Scientific Inc., Germany) for disease development. The moisture of paper towels was maintained by sprinkling water on the paper towel, daily (Sharma and Pande, 2013). At 7 days after incubation, disease scoring was carried out using a 1-to-9 scale (Nene et al., 1981; Pandey et al., 2020), where 1 = Immune (I), >1 and ≤ 3 = Resistant (R), >3 and ≤ 5 = Moderately resistant (MR), >5 and ≤ 6 = Moderately Susceptible (MS), >6 and ≤ 8 = Susceptible (S), and >8 or 9 = Highly Susceptible (HS). To verify Koch's postulates, MP was re-isolated from the inoculated plants showing symptoms of DRR.

2.4. Molecular characterization of the MP1 and MP2 isolates

The identity of MP1 (used for screening of accessions in India) and

Table 1
Phenotypic and morphological characters of MP isolates from different agro-climatic regions.

Isolates ID	Location[a]	Phenotypic and morphological features					Disease score (1–9 Scale)
		Colony color	Growth pattern[b]	Shape of Sclerotia	Number of sclerotia[$]	Sclerotia diameter (μM)[c]	
MP1	Hyderabad	Greyish black	+	Oblong	189.3	113.2	9.0
MP2	Yezin	Blackish grey	++	Round	208.4	84.2	–
MP3	Bhubaneswar	Greyish white	+++	Round	165.0	93.6	6.7
MP4	Ludhiana	Blackish grey	+	Round	181.0	85.5	8.4
MP5	Palampur	Greyish white	++	Round	174.3	83.8	7.7
MP6	Mongrakalan	Black	++	Oblong	141.7	78.6	6.7
MP7	Jodhpur	Blackish grey	+	Oblong	160.0	76.0	7.0
MP8	Bijwaria	Blackish grey	+	Oblong	178.0	87.7	8.3
MP9	Rampur	Black	+++	Round	168.3	87.8	8.0
MP10	Masa	Greyish white	+++	Round	146.7	86.3	7.0
MP11	Amravati	Black	+++	Round	169.7	90.4	8.3

[a] All locations were from India except Yezin (Myanmar).
[b] Formation of septum in the branch near the origin in all isolates, +: Less feathery, ++: Moderate Feathery, +++: More Feathery, $average of three replicates per9 mm disc.
[c] Average of 10 sclerotia, MP: *Macrophomina phaseolina*.

MP2 (used for screening of accessions in Myanmar) isolates as MP that were relatively more pathogenic were also confirmed by sequencing the ITS region. The ITS region (ITS1, 5.8S, and ITS2) of the nuclear rDNA operon of both isolates were amplified and sequenced. The gDNA (genomic DNA) from each MP isolate was extracted using the CTAB method (Moller et al., 1992) and quantified with a NanoDrop1000 spectrophotometer (Thermo Scientific, USA). The rDNA gene cluster was amplified by PCR, using universal primer pairs ITS1/ITS4 for isolate MP1 and ITS4/ITS5 for isolate MP2 (White et al., 1990). The amplified PCR products of each isolate were separated by electrophoresis on a 2% agarose gel, and the obtained bands were excised and purified (UniPro Gel extraction kit) for sequencing (Macrogen, Inc., Korea). BLASTn was used to match the sequence of each isolate with known sequences of MP strains available on the public database Genbank.

2.5. Plant materials

The 296 mungbean mini-core accessions used for the paper towel experiment were obtained from the World Vegetable Center South Asia, Hyderabad. These accessions were developed from a core collection of 1481 mungbean accessions at the World Vegetable Center, Taiwan in 2015 to assess potential resistance to both biotic and abiotic stresses. These accessions have a diverse origin with Africa, Europe, Central America, North America, Oceania, and the Pacific, South Asia, South America, Southeast Asia, and Southwest Asia (Schafleitner et al., 2015).

2.6. Evaluation of mungbean mini-core accessions for DRR resistance

In the preliminary experiment, 296 mungbean accessions were screened against isolate MP1 for DRR resistance using the paper towel method as previously described, at World Vegetable Center South Asia, Hyderabad (India). Twenty-nine resistant accessions identified in the preliminary test were re-evaluated by the paper towel method to see the consistent resistance response. Eighteen accessions consistent with resistance response in the repeated paper towel experiment were further evaluated for DRR resistance in a glasshouse in Hyderabad through the sick pot method (Choudhary et al., 2011). A subset of 30 accessions showing resistant (27) and susceptible (3) reactions in the preliminary paper towel experiment in Hyderabad was selected from 296 mini-core accessions. These accessions were evaluated for DRR resistance in the field at Food Legume Research Section, Department of Agricultural Research (DAR), Yezin, Myanmar. The accessions consistent with resistance response in the glasshouse (Hyderabad) and field (Yezin) trials have also been evaluated against nine different isolates of MP from India (Table 1) through the paper towel method in Hyderabad. All the experiments were conducted from 2017 to 2019.

2.6.1. Evaluation of mungbean accessions for DRR resistance by the paper towel method

Mungbean seeds of 296 mini-core accessions were sown in plastic pro trays containing sterilized black-sandy soil mixtures, separately. To raise the seedlings, these pro trays were kept at 25 °C for 8 days in the glasshouse. The grown seedlings were uprooted at 8-day-old, and roots were rinsed with tap water. The uprooted seedlings were kept by accession separately in polyethylene bags (sterilized) for transportation to the laboratory for the paper towel experiment. The isolate MP1 was used for the preparation of the inoculum. The paper towel experiment was conducted using the same methodology as previously described in the pathogenicity section. The prepared paper towels were labelled with each accession and arranged in a completely randomized design (CRD) with three replications in lots of ten in a tray. The trays were kept at 35 °C and with a photoperiod of 12 h inside a BOD incubator for disease development. The moisture of paper towels was maintained as previously described. At 7 days after incubation, disease scoring was carried out using a 1-to-9 rating scale. The resistant accessions obtained through the experiment were re-evaluated using the same procedure to see their consistent resistance response in the repeated experiment.

2.6.2. Evaluation of resistant mungbean accessions for DRR resistance by the sick pot method

The resistant mungbean accessions (18) obtained through repeated paper towel experiments were evaluated in sick pots in a glasshouse in Hyderabad to confirm their resistance levels. The pots (6-inch-diameter), containing sterilized black-sandy soil mixture (2g) and inoculated with isolate MP1 (50 g/kg soil) grown on sorghum grains (Choudhary et al., 2011) were used for experiments. Before commencement of the experiment, to confirm whether the pathogen inoculated in pots was pathogenic or nonpathogenic on the host, at 5 days after sick pot preparation, pots were sown with a DRR susceptible mungbean variety VC3960-88 at the rate of 10-seeds/pot. These pots were kept in a glasshouse (32 ± 2 °C). Once the mortality of plants in susceptible check reached above 90%, these pathogen inoculated pots (~105 microsclerotia/g of soil) were selected for the evaluation of resistant accessions identified through the repeated paper towel method.

In the individual pot, ten seeds of each resistant accession and susceptible check were sown, separately. These pots were labelled with each accession and accessions were arranged in a randomized complete block design (RCBD) with three replications. Control pots included the susceptible check sown in soil free from the pathogen inoculum. The soil moisture was maintained at 60% water holding capacity. Once the mortality of plants in susceptible check reached above 90% (45 days after sowing), numbers of DRR symptomatic plants in each accession was assessed and recorded in a field notebook. Percent disease incidence

(PDI) of each accession due to DRR was calculated by the formula, PDI = Total number of DRR symptomatic plants/Total number of plants × 100 (Cooke, 2006). Based on the range of PDI, the test accessions were categorized as highly resistant (free from DRR), resistant (≤10.0% incidence), moderately resistant (10.1–20.0% incidence), moderately susceptible (20.1–30%), susceptible (30.1–50.0% incidence), and highly susceptible (>50% incidence).

2.6.3. Field evaluation of selected mungbean accessions for DRR resistance in Yezin, Myanmar

A subset of 30 mungbean accessions selected from 296 mini-core accessions based on *in vitro* results was re-evaluated for DRR resistance under the field conditions, in Yezin (Myanmar) in the post-rainy season of y 2018 and 2019, under natural MP inoculum pressure. Two local accessions such as Yezin 11 and Yezin 14 were also included with 30 accessions in the field trial. The field experiment was conducted at Food Legume Research Section, Department of Agricultural Research (DAR), Yezin, Myanmar (N 19°50′11.95′, E 96°16′19.62′, Elevation: 120.2 m) under a no-tillage system without irrigation.

The experimental units consisted of a 4 rows plot of 5-m-long. In total, 90 seeds (2-seeds/burrow) per accession were planted in 5-m-long rows spaced 45 cm apart with the plant to plant distance of 10 cm. In the field, accessions were arranged in an alpha lattice design with three replications. Once the mortality started in susceptible check, the total number of DRR symptomatic plants was recorded in each accession. In each year of the experimental trial, at 25 days after sowing, a manual hand weeding was done for weed management. Also, to overcome other confounding effects by pests and diseases in research plots, appropriate management practices were carried out following the mungbean field manual of Mbeyagala et al. (2017). The wilting of plants was examined every day after sowing. Percent disease incidence (PDI) of each accession due to DRR was calculated by the formula, PDI = Total number of DRR symptomatic plants/Total number of plants × 100 as suggested by Cooke (2006) when the first symptom started to appear. The final reporting of disease incidence was carried out until the harvesting time.

2.7. Re-evaluation of resistant mungbean accessions against nine MP isolates from India

The resistant mini-core accessions, such as VI001509AG, VI001244AG, and VI001400AG with consistent resistance response in the glasshouse and field experiments, were screened against nine MP isolates from India using the paper towel method as previously described, to see the variance in resistance. A susceptible check (VC3960-88) was also included in the experiment. For each isolate, the experiment was carried out separately with three replicates in a CRD. Finally, after disease development, at 7 days after incubation, scoring of symptomatic seedlings of each accession was carried out using a 1-to-9 scale.

2.8. Data analyses

Each experiment was repeated and carried out with three replicates. The replicate-wise values were used for statistical analysis. Prior to the analysis, square root and arcsine transformation were applied for disease score and percent disease incidence, respectively. To test the significance of the experiments, combined and trial-wise ANOVA (analysis of variance) were conducted with accessions and trial × accessions effects using the MIXED procedure of SAS (SAS Institute Inc, 2018). In the MIXED procedure, individual trial residual variances were modeled into combined analysis using the REPEATED statement. Best Linear Unbiased Estimators (BLUEs) were estimated for main and interaction effects from combined analysis of variance. Additionally, line means were separated using Fisher's protected Least Significance Test (LSD).

A set of 15 accessions, common in both glasshouse (sick pot1 & sick pot2) and field (2018 & 2019) experiments were subjected to the GGE biplot analysis for DRR disease incidence. GGE biplot analysis was conducted to determine the resistance stability of genotypes across test environments/trials (Yan and Falk, 2002). Four environments (trials) and fifteen genotypes (accessions) were used in biplot analysis and a site regression model was used to visualize the trial × accession patterns to identify resistant accessions across the trials (Yan and Kang, 2003). The GGE biplot was created by plotting the PDI of the Genotype (accessions) (G) and the environments (trials) (E) as first principal components (PC1) on X-axis against their respective PDI for the second principal component (PC2) on Y-axis (Yan and Kang, 2003). These components were derived from the single value decomposition of the environment-centered data. In the same plot G and E were presented. Each G and E was defined by their scores on the two principal components. To evaluate the correlation among the environment angles between the environments, vectors were used. The vector length represents the genotypic variability in the respective environment. To evaluate the genotypes' stability, the AEC (average environment coordinate) was plotted by taking the mean scores of PC1 and PC2 for environments. The mean performance of the genotype was determined using a performance line passing through the origin of the biplot. In biplot, the arrow on the performance line presents a higher mean disease incidence of a genotype, i.e., higher susceptibility.

Besides, to identify the relationship between trials, Spearman's rank correlation was performed by comparing the PDI of 15 accessions common across trials and locations, using PROC CORR procedure (SAS Institute Inc, 2018).

3. Results

3.1. Cultural and morphological variability

The studies on cultural and morphological characteristics revealed that all MP isolates showed variable degrees of growth pattern, colony color, shape, size, and the number of sclerotia. On the PDA plate, colony color varied between the isolates (Table 1), which were either black (MP6, MP10, MP11), blackish grey (MP2, MP4, MP7, MP8; Fig. 1b and d), greyish white (MP3, MP5, MP10), or greyish black (MP1). Isolates formed round or oblong-shaped sclerotia that were either dark-brown or black. The isolate MP2 produced the maximum number of sclerotia (208.4 sclerotia/9 mm disc) followed by isolates MP1 (189.3 sclerotia/9 mm disc) and MP4 (181/9 mm disc) (Fig. 1a and c). Other isolates demonstrated a significant ($P < 0.001$) variation in the sclerotia number in a range of 141.7–174.3/9 mm disc. Besides, MP1 recorded a larger size of sclerotia (113.2 μm) followed by isolate MP3 (93.6 μm), while MP7 showed a smaller sclerotia size (76.0 μm). All tested isolates were pathogenic on mungbean susceptible genotype. The DRR disease severity scores ranged from 6.7 to 9.0 (on 1-to-9 scale), and the effect of isolates on disease severity scores was significant ($P < 0.001$). The isolate MP1 was more pathogenic, hence it was selected for the screening of mini-core accessions in Hyderabad.

3.2. Molecular characterization of the MP1 and MP2 isolates

The isolates of MP used for screening of 296 mungbean accessions and resistant accessions in Hyderabad (MP1) and Yezin (MP2) were also identified by sequencing the ITS region. Results from the BLAST analysis of ITS sequences revealed that MP1 and MP2 isolates were belonging to *M. phaseolina*. In BLASTn search, sequences of isolate MP1 exhibited 99.9% resemblance with the sequences of MP isolates isolated from other hosts, such as common bean (KU831500.1), cowpea (KF951783.1), mungbean (KF951636.1), potato (KU721993.1), urdbean (KF951637.1), and cotton (KX270356.1). Likewise, the isolate MP2 showed >99% resemblance with the ITS sequences of MP isolates isolated from other hosts, for instance, mandarin (MH168332.1), cowpea (MK926448.1), broad bean (MH323406.1), common bean (KT768131.1), *Mentha* species (MT186826.1), and Spider lily

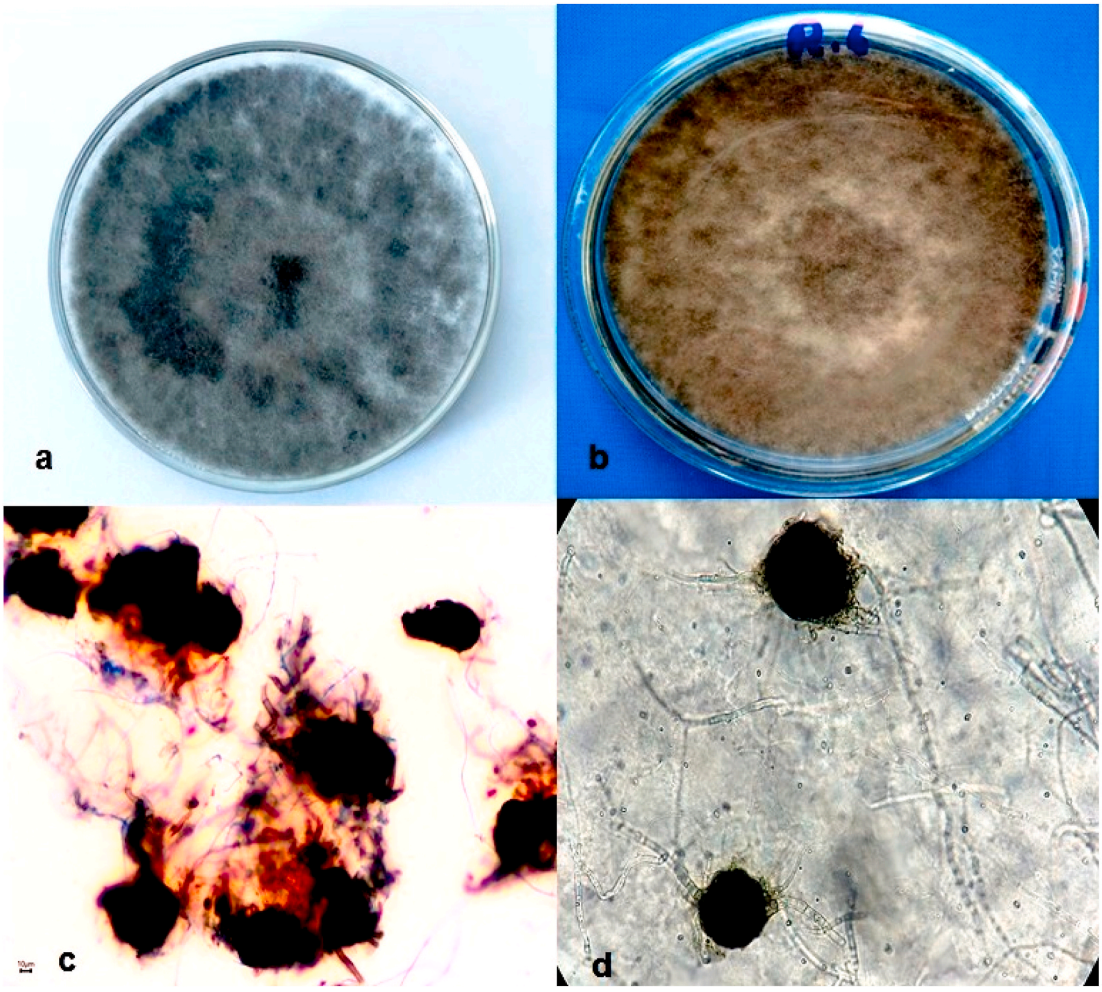

Fig. 1. Cultural and morphological characteristics of DRR pathogen, *Macrophomina phaseolina.* Figures a (MP1-Hyderabad) & b (MP2-Yezin) are showing pathogen on the agar plate, and c (MP1) & d (MP2) are showing microsclerotia of MP.

(MK408587.1) in the BLAST search. The nucleotide sequences of isolates MP1 and MP2 were submitted in GenBank (NCBI) under the accession numbers MN006689 and MT634693, respectively.

3.3. Evaluation of mungbean accessions for DRR resistance by the paper towel method

Evaluation of 296 mungbean mini-core accessions against MP1 isolate showed a significant variation ($P < 0.0001$) in their disease reactions (Fig. 2). The DRR disease reactions of resistant and susceptible accessions through the paper towel method are shown in Fig. 3a. Out of the 296 accessions screened, the accession VI001509AG was free from DRR symptoms with mean disease scores 'one'. In 295 remaining accessions, 28 accessions were resistant, 124 were moderately resistant, 62 were moderately susceptible, 75 were susceptible, and six were highly susceptible with mean disease scores ranged between 1.1 and 3.0, 3.1 and 5.0, 5.1 and 6.0, 6.1 and 8.0, and 8.1 and 9.0, respectively (Fig. 2). In a repeated paper towel experiment with 29 resistant accessions, 18 accessions were identified as resistant, six moderately resistant, and five were moderately susceptible, and the ANOVA exhibited a significant ($P < 0.0001$) variation in disease scores (Table 2).

3.4. Evaluation of resistant mungbean accessions for DRR resistance by the sick pot method

Results from glasshouse experiments revealed that there was no significant variation ($P > 0.05$) observed between the repeated experiments sick pot1 and sick pot2 (Table 3). A significant difference for percent disease incidence was observed between resistant and susceptible accessions ($P < 0.0001$). Out of the 18 accessions screened, based on the percent disease incidence, ten accessions were resistant and eight were moderately resistant in sick pot 1, and 13 accessions were resistant and five were moderately resistant in sick pot 2 (Table 3). Data of the repeated glasshouse experiments revealed that accessions VI000766BG, VI001244AG, VI001268BG, VI001282AG, VI001400AG, VI001490AG, VI001509AG, VI001535BG, and VI003699B-BG performed consistent resistance in sick pot 1 and 2. These accessions had lower DRR incidence (≤10.0%) than the susceptible check (VC6930-88), which showed 91.36% disease incidence in sick pot 1 and 96.20% in sick pot 2. The disease reaction of resistant and susceptible accessions is shown in Fig. 3b.

3.5. Field evaluation of selected mungbean accessions for DRR resistance in Yezin, Myanmar

Out of the 30 mungbean mini-core accessions screened, in 2018, two accessions were highly resistant with absence of DRR symptomatic plants, and nine were resistant with a percent disease incidence of ≤10.0. In 19 remaining accessions, two were moderately resistant, two were moderately susceptible, four were susceptible, and 11 were highly susceptible with percent disease incidence ranged between 10.1% and 20%, 20.1% and 30%, 30.1% and 50%, and 50.1% and 100%,

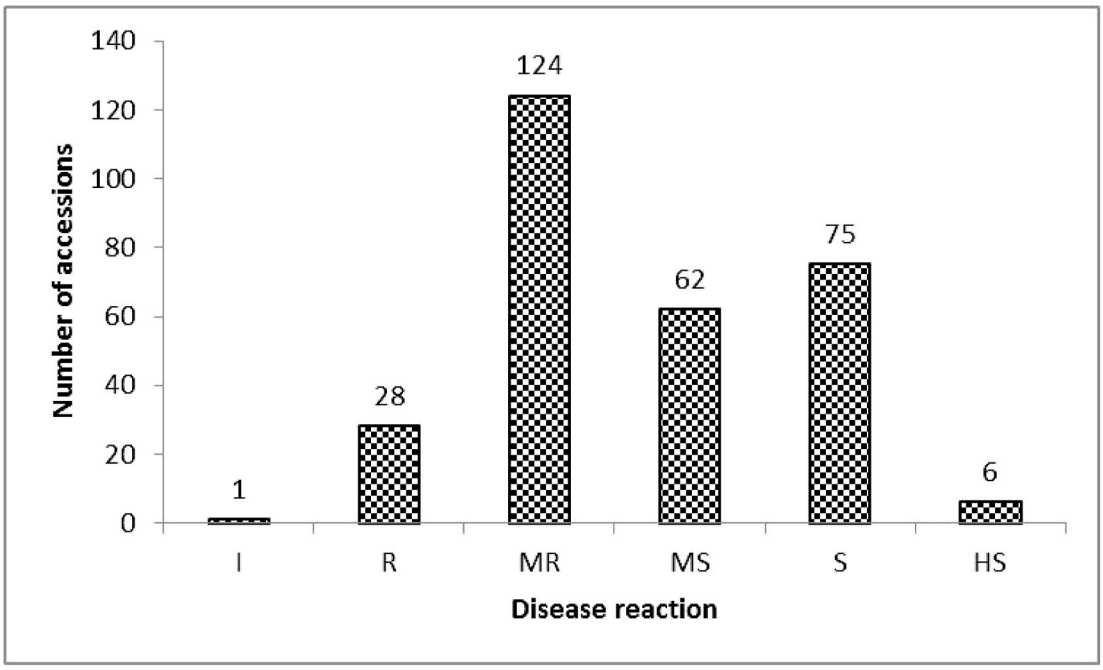

Fig. 2. Disease reaction of mungbean mini-core accessions against DRR. Disease score was rated on a 1-to-9 scale where 1 = Immune and 9 = Highly susceptible, total accessions 296, I: Immune, HR: Highly Resistant, R: Resistant, MR: Moderately Resistant, MS: Moderately Susceptible, S: Susceptible, HS: Highly Susceptible, $P < 0.0001$.

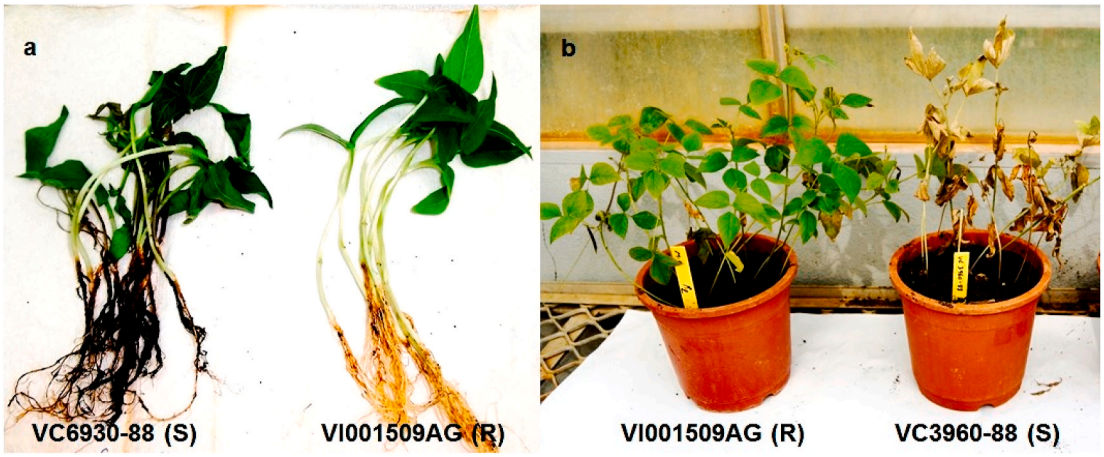

Fig. 3. Mungbean accessions showing susceptible (S) and resistant (R) reactions against DRR in paper towel (a) and sick pot (b) experiments at the WorldVeg South Asia, Hyderabad, India.

respectively (Table 3). In 2019, four accessions were highly resistant (free from DRR) and 21 were resistant (≤10.0%). In five remaining accessions, one each was moderately resistant, moderately susceptible, and susceptible, whereas two accessions did not germinate (Table 3). Local varieties Yezin 11 and Yezin 14 were susceptible in 2018, while resistant in 2019. The individual ANOVA exhibited a significant variation in percent disease incidence of DRR among the mini-core accessions in 2018 ($P < 0.003$) and 2019 ($P > 0.0006$) of evaluation trials.

3.6. GGE biplot and correlation analysis of selected mungbean accessions

The GGE biplot analysis of the 15 accessions common in Hyderabad and Yezin explained 97.24% of the total variation. Respective PC1 and PC2 accounted for 91.69 and 5.55% of variations, respectively (Fig. 4). Out of the 15 accessions, seven accessions distanced farther from the biplot origin created a heptagon. The accessions placed at the vertices of the heptagon contributed the most to the interaction, i.e., those with the lowest or highest DRR incidence. The accessions VI001244AG, VI001509AG, and VI001400AG located farthest to the left side of the biplot origin endorsed their DRR resistance across the trials. These three accessions had higher levels of stability and lower disease incidence compared with all the other accessions. The accession VI000319AG, VI000818BG, VI001268BG, and VI001548AG were more susceptible to DRR by being farthest on the right side of the biplot origin on the performance line (Fig. 4). The biplot analysis showed that the trial conducted in 2018 in Yezin (Myanmar) had a longer vector than other trials signifying that this was the environment that discriminated genetic variability of the accessions. However, the trials conducted in 2019 in Yezin (Myanmar), and in the glasshouse (sick pot 1 and sick pot 2) in Hyderabad had smaller vectors, representing they were less discriminative of accessions.

AEC was created on the biplot to evaluate the test-trial and stability

Table 2
DRR disease reaction of identified resistant mungbean accessions in the repeated paper towel method.

Mungbean accessions	Origin	Paper towel 1		Paper towel 2	
		Disease score	Reaction category	Disease score	Reaction category
VI000203B-BR	Afghanistan	2.8	R	1.3	R
VI000319AG	Pakistan	1.6	R	1.9	R
VI000732AG	India	2.6	R	1.8	R
VI000764AG	India	2.5	R	2.6	R
VI000766BG	India	2.1	R	2.4	R
VI000805BG	India	3.0	R	4.4	MR
VI000815BG	India	2.2	R	3.6	MR
VI000818BG	India	2.5	R	1.7	R
VI000981BG	Philippines	1.4	R	4.5	MR
VI001244AG	Philippines	1.6	R	1.7	R
VI001268BG	India	1.6	R	2.2	R
VI001282AG	India	1.5	R	2.1	R
VI001284AG	India	2.6	R	1.7	R
VI001400AG	India	2.0	R	3.0	R
VI001403BR	India	1.6	R	5.3	MS
VI001412AG	India	3.0	R	3.9	MR
VI001419BG	India	1.9	R	2.7	R
VI001482BG	India	2.7	R	5.1	MS
VI001490AG	Iran	3.0	R	3.0	R
VI001509AG	Pakistan	1.0	I	1.1	R
VI001535BG	India	2.4	R	2.4	R
VI001548AG	India	2.9	R	2.5	R
VI001576BG	India	1.7	R	5.5	MS
VI002529B-BL	Thailand	2.4	R	2.7	R
VI002587AG	Australia	2.8	R	3.1	MR
VI003070AG	India	2.5	R	4.4	MS
VI003699B-BG	India	2.5	R	3.0	R
VI004024AG	Australia	1.3	R	6.4	MS
VI004811BG	India	2.9	R	3.5	MR
VI002859BG	Iran	8.2	S	7.6	S
CV%		14.89		6.12	
LSD (5%)		1.23		0.18	
R-Square		0.87		0.96	
MSS		7.10[a]		0.53[a]	

[a] Significant at 1% probability level, CV: Coefficient of variation, LSD: Least significant difference, MSS: Mean sum of square, DRR disease score was rated on a 1-to-9 rating scale, where 1 = Immune and 9 = Highly susceptible, I= Immune, R: Resistant, MR: Moderately resistant, MS: Moderately susceptible, S: Susceptible, HS: Highly susceptible.

of the accessions. In Fig. 4, the circles signify coordinates equivalent to the average coordinates of the four marker points for trials. The axis (blue) passed through the biplot origin and in the AEC direction, labelled the AECa (AEC absicca), and on the AECa, an arrow pointed towards the high DRR incidence direction. Three accessions (VI001244AG, VI001509AG, and VI001400AG) at the left side of the Y-axis and nearer to the performance line had stable resistance across trials. However, accessions toward the right side of the AEC coordinate had a higher DRR incidence (Fig. 4).

In Spearman's rank correlation analysis of glasshouse and field PDI data, a significant positive correlation (r = 0.4) was found between trials of Myanmar (Yezin) 2018 & 2019 and Myanmar (Yezin) 2018 & sick pot 2 concerning levels of DRR incidence. However, a negative correlation (r = −0.13) was observed between the trials of Myanmar (Yezin) 2019 and sick pot 1 (Fig. 5). There was less correlation (r = 0.13) found between trials of Myanmar (Yezin) 2018 and sick pot 1, while no correlation (r = 0.05) was observed between Myanmar (Yezin) 2019 and sick pot 2. Besides, a positive correlation (r = 0.34) was also observed between sick 1 and sick 2 trials. Pooled analysis (ANOVA) for percent disease incidence data of 15 accessions common in glasshouse and field experiments showed a significant ($P < 0.0001$) variation among the tested accessions for DRR resistance (Table 4).

Table 3
DRR disease reaction of identified resistant mungbean accessions in the glasshouse and in the field.

Mungbean accessions	Sick pot-Hyderabad, India		Field-Yezin, Myanmar	
	Sick pot 1 (2018)	Sick pot 2 (2018)	2018	2019
	PDI (Reaction category)	PDI (Reaction category)	PDI (Reaction category)	PDI (Reaction category)
VI000203B-BR	13.01 (MR)	13.01 (MR)	×	0.00 (HR)
VI000319AG	16.35 (MR)	16.35 (MR)	81.41 (HS)	4.23 (R)
VI000732AG	16.35 (MR)	10.00 (R)	13.29 (MR)	0.00 (HR)
VI000764AG	13.01 (MR)	13.01 (MR)	16.6 (MR)	1.12 (R)
VI000766BG	10.0 (R)	10 (R)	88.04 (HS)	9.78 (R)
VI000805BG	×	×	×	2.21 (R)
VI000815BG	×	×	0.00 (HR)	2.40 (R)
VI000818BG	20.0 (MR)	13.01 (MR)	65.83 (HS)	31.00 (S)
VI000981BG	×	×	86.52 (HS)	3.79 (R)
VI001244AG	6.67 (R)	6.67 (R)	3.77 (R)	2.31 (R)
VI001268BG	10 (R)	6.67 (R)	99.99 (HS)	16.16 (MR)
VI001282AG	6.76 (R)	6.67 (R)	43.06 (S)	8.85 (R)
VI001284AG	4.58 (R)	13.01 (MR)	28.48 (MS)	27.41 (MS)
VI001400AG	10.00 (R)	10.00 (R)	3.88 (R)	0.00 (HR)
VI001403BR	×	×	×	0.92 (R)
VI001406BG	×	×	23.70 (MS)	×
VI001412AG	×	×	62.21 (HS)	2.57 (R)
VI001419BG	13.01 (MR)	10.00 (R)	73.43 (HS)	0.39 (R)
VI001482BG	×	×	9.74 (R)	0.03 (R)
VI001490AG	10.0 (R)	4.58 (R)	0.92 (R)	×
VI001509AG	4.58 (R)	4.58 (R)	2.60 (R)	0.17 (R)
VI001535BG	6.76 (R)	4.58 (R)	49.80 (S)	7.31 (R)
VI001548AG	13.01 (MR)	13.01 (R)	95.68 (HS)	0.32 (R)
VI001576BG	×	×	40.18 (S)	0.30 (R)
VI002529B-BL	20.00 (MR)	1.20 (R)	0.33 (R)	2.69 (R)
VI002587AG	×	×	0.00 (HR)	0.59 (R)
VI002859BG	×	×	6.51 (R)	3.66 (R)
VI003070AG	×	×	73.25 (HS)	1.66 (R)
VI003220AG	×	×	55.37 (HS)	×
VI003456AG	×	×	94.25 (HS)	×
VI003699B-BG	10.00 (R)	10.00 (R)	31.57 (S)	×
VI004024AG	×	×	7.98 (R)	0.00 (HR)
VI004811AG	×	×	0.63 (R)	0.23 (R)
VC6930-88	91.36 (HS)	96.20 (HS)	×	×
Yezin 11	×	×	82.5 (HS)	1.3 (R)
Yezin 14	×	×	29.2 (MS)	0.3 (R)
SE	0.0037	0.004	0.02	0.002
Probability (F > 0.05)	<0.0001[a]	<0.0001[a]	>0.003[a]	>0.0006[a]
Chi-square value	×	×	0.60	0.70
F-Value	22.56	22.75	5.67	2.88

[a] Significant, ×: not performed, SE: Standard error, PDI: Percent disease incidence, HR: Highly Resistant,R: Resistant, MR: Moderately resistant, MS: Moderately susceptible,S: Susceptible, HS: Highly susceptible.

3.7. Re-evaluation of resistant mungbean accessions against nine MP isolates from India

The ANOVA revealed a significant difference in the disease reaction ($P < 0.0001$, CV%: 6.1, R2: 0.95, F-value: 45.4, LSD: 0.17, MSS: 16.069) among the resistant and susceptible accessions. Among the test accessions, VI001509AG and VI001400AG were resistant against all nine MP isolates, while VI001244AG was resistant against isolates MP5, MP6, and MP7, moderately resistant against MP3, MP4, MP8, and MP9, while moderately susceptible against MP10 and MP11 isolates (Fig. 6).

4. Discussion

Dry root rot is spreading rapidly in mungbean in Asian countries due to enhanced water stress during the cropping period because of irregular and reduced rainfall and increase temperature. The pathogen infects a wide range of host plants and survives longer in the soil. There are few

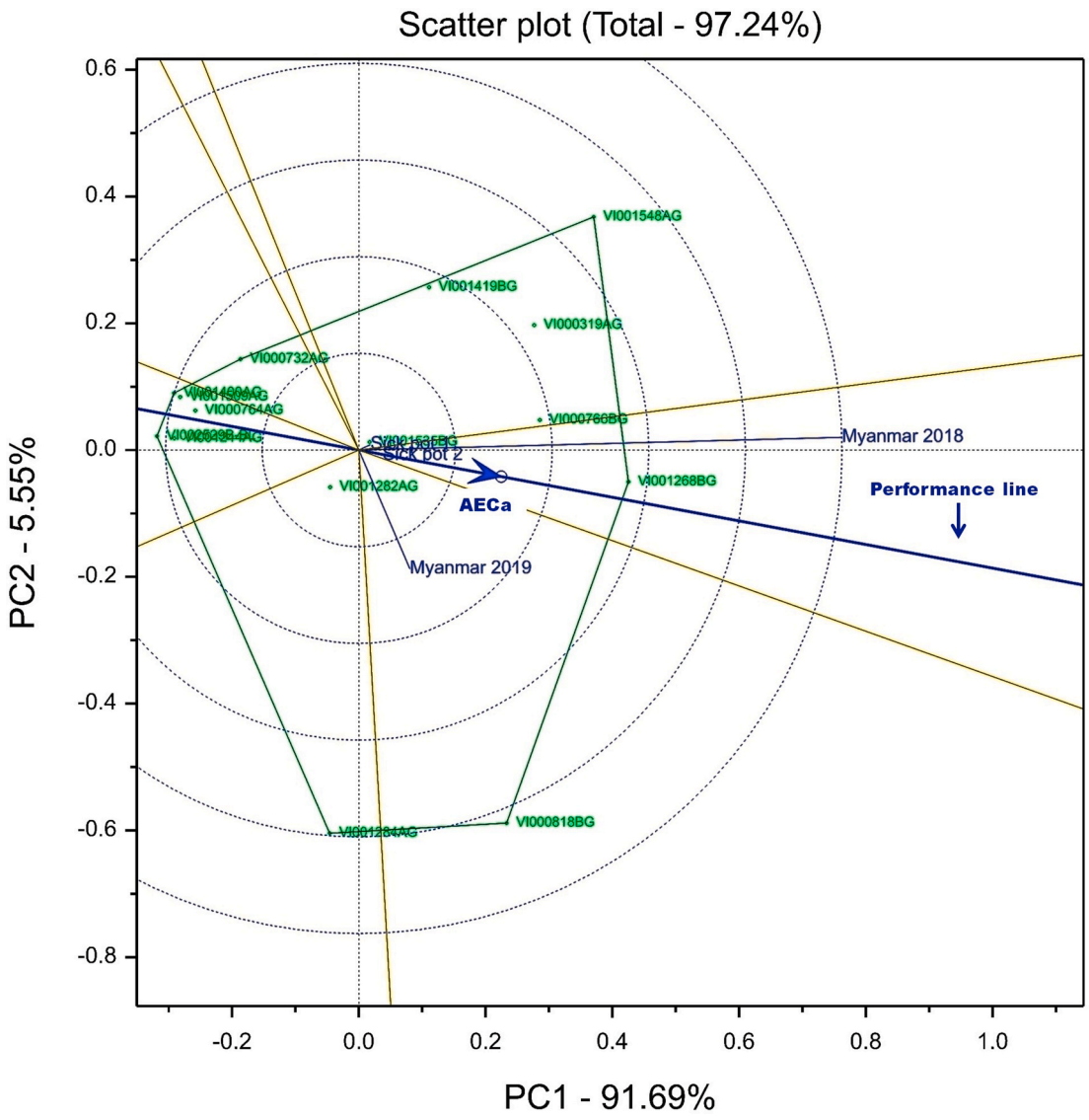

Fig. 4. GGE biplot of first and second principal components (PC1 and PC2, respectively) based on DRR percent disease incidence of 15 mungbean mini-core accessions in glasshouse (Sick pot 1 & 2, Hyderabad) and field (2018 & 2019, Yezin) experiments. AECa: abscissa of the "Average Environment Coordination" axis, which connects the origin with the environmental average.

sources of resistance available against DRR of mungbean, but they are not durable due to pathogenic variability of MP (Gupta et al., 2012) and also polyphagous nature of the pathogen (Kumar et al., 2017). Over the past years, isolates of MP isolated from leguminous crops were characterized using morphological and molecular techniques (Babu et al., 2007; Khan, 2008; Khan et al., 2017). In this study, each isolate of MP has also been characterized using cultural and morphological characteristics.

Cultural studies of each isolate revealed that morphologically, all MP isolates were similar to each other with variable structure and size of microsclerotia. The sclerotia size of each isolate varied from round to oblong. As compared to the other isolates, the isolate MP2 produced the maximum number of sclerotia, and MP1 isolate demonstrated a bigger size of sclerotia. Historically, sclerotia helped in the survival of the pathogen, MP. The isolate of MP which produces more sclerotia can be more pathogenic than the isolates that produced fewer sclerotia and cause higher seedling mortality (Sharmishtha et al., 2004). Besides, the disease severity is also directly correlated with the population of viable sclerotia available in the soil (Sundravadana et al., 2012), for example, disease severity in sesame increased due to higher inoculum density of MP in the soil (Sankar, 1994). Similarly, in the present study, MP1 and MP2 isolates from respective Hyderabad and Yezin produced a maximum number of sclerotia and could be more virulent isolates as reported for MP1 during the pathogenicity test. In the present study, all the eleven MP isolates exhibited a variable size of sclerotia which supported the findings of earlier researchers who reported the variation in sclerotia size among the different MP isolates (Suriachandraselvan and Seetharaman, 2003; Tandel et al., 2012).

In many developing countries, mungbean growers use carbendazim as a seed treatment to manage the disease (Kumari et al., 2015), but the cost and use of fungicides are too expensive and not economical at the farmer level. In contrast, progress in the deployment of DRR resistant varieties would be efficient, practically feasible, and would be compatible with other components of disease management. The mini-core collections from other crops, such as chickpea, sorghum, and pigeon pea (Pande et al., 2006; Sharma et al., 2010, 2012) were evaluated against multiple diseases, but needed in mungbean. Therefore, the present studies were undertaken to identify sources of resistance to DRR from a mungbean mini-core collection. In the present study, 296 mini-core accessions were screened against MP by the paper towel method under controlled conditions. The screening revealed that out of the 296 accessions, 29 were resistant, and among the 29 accessions, 18

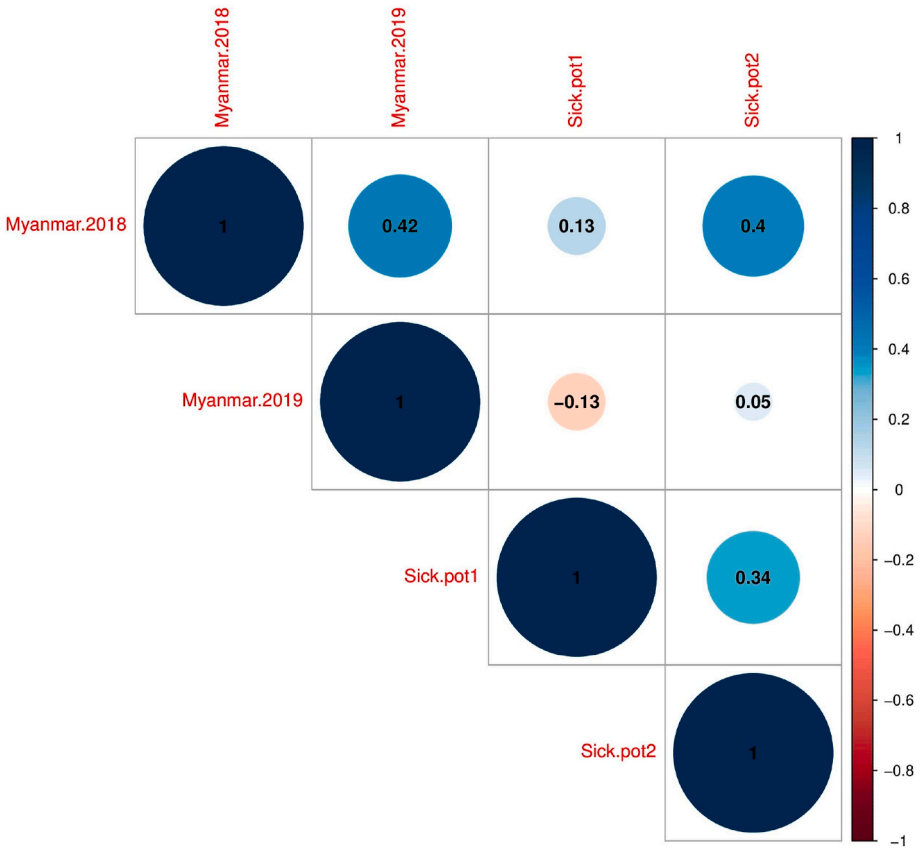

Fig. 5. Spearman's rank correlations (r) showing stability and comparison of 15 mini-core accessions common in glasshouse and field for DRR resistance across the trials. The numeric values depicted here are correlation coefficient (r).

Table 4
Pooled analysis of variance for percent DRR incidence of 15 mungbean accessions common in glasshouse and field evaluation trials.

Effect	Num DF	Den DF	F value	Pr > F
Trial	3	12	21.79	<.0001
Rep (Trial)	7	9.51	1.57	0.2543
Accession	14	20.2	6.17	0.0001
Trial × Accession	42	28.7	2.62	0.0040
Estimates of random effects (Z-value)				
	Estimates	Standard Error	Z Value	Pr > Z
Myanmar 2018	0.05978	0.02799	2.14	0.0164
Myanmar 2019	0.02180	0.006503	3.35	0.0004
Sick pot 1	0.01584	0.004269	3.71	0.0001
Sick pot 2	0.02048	0.005916	3.46	0.0003

Num DF: Numerator degrees of Freedom, Den DF: Denominator (error) degree of Freedom.

were identified with consistent resistance in the repeated experiment.

Researchers reported that the paper towel method was very useful for screening more germplasm for the identification of resistant accessions as it saved assets and time extent (Sharma et al., 2015). However, the paper towel method has one drawback that host × pathogen interaction time is limited, as the seedlings' roots are exposed to the pathogen for a limited period. Consequently, resistant accessions obtained through the paper towel method should be validated in sick pot assay or in the field conducive for DRR, as both screening methods offer longer periods (60–80 days) for host × pathogen × environment interaction. Therefore, in the present investigation, 18 DRR-resistant accessions obtained through the repeated paper towel method were further evaluated in sick pots to confirm their resistance levels. Out of the 18 accessions, nine accessions showed consistent resistance in the glasshouse with a higher number of plants' survival than the susceptible check.

Identification of resistant mungbean accessions against DRR with consistent performance across different locations is useful for future breeding programs and IDM (integrated disease management). The presence of genotype × environment interaction creates paradox during the screening of accessions in multi-environment and assessment against the disease (Das et al., 2019). Therefore, in the present study, a subset of 30 mungbean mini-core accessions were evaluated for DRR resistance at the field in Yezin, Myanmar where DRR was severe in earlier cropping period. Out of the 30 accessions, ten accessions, such as VI000815BG, VI001244AG, VI001400AG, VI001482BG, VI001509AG, VI002529B-BL, VI002587AG, VI002859BG, VI004024AG, and VI004811AG were resistant in both years of evaluation trials. Therefore, these accessions can be recommended for cultivation in the DRR problematic areas in Myanmar. ANOVA reflected the prevailing effect of environment followed by G × E interactions toward DRR incidence of the screened mungbean accessions. In the present study, higher levels of DRR incidence in susceptible accessions in evaluation trials of Yezin indicated adequate disease pressure under the natural conditions in both years.

Mini-core accessions VI001509AG, VI001244AG, and VI001400AG were consistently resistant in both glasshouse and field. The stable resistant performance of the accessions in both glasshouse and field conditions is evident from the correlation analysis. Spearman's rank correlation analysis of 15 mini-core accessions common in Hyderabad and Yezin revealed positive and negative correlations across the trials or locations. In a pooled analysis of these 15 accessions for DRR, G × E interaction was significant, hence for each experiment data were analyzed separately.

Analysis of the stability of the 15 mungbean accessions for DRR resistance using the GGE biplot method showed that accessions VI001244AG, VI001509AG, and VI001400AG were considered stable

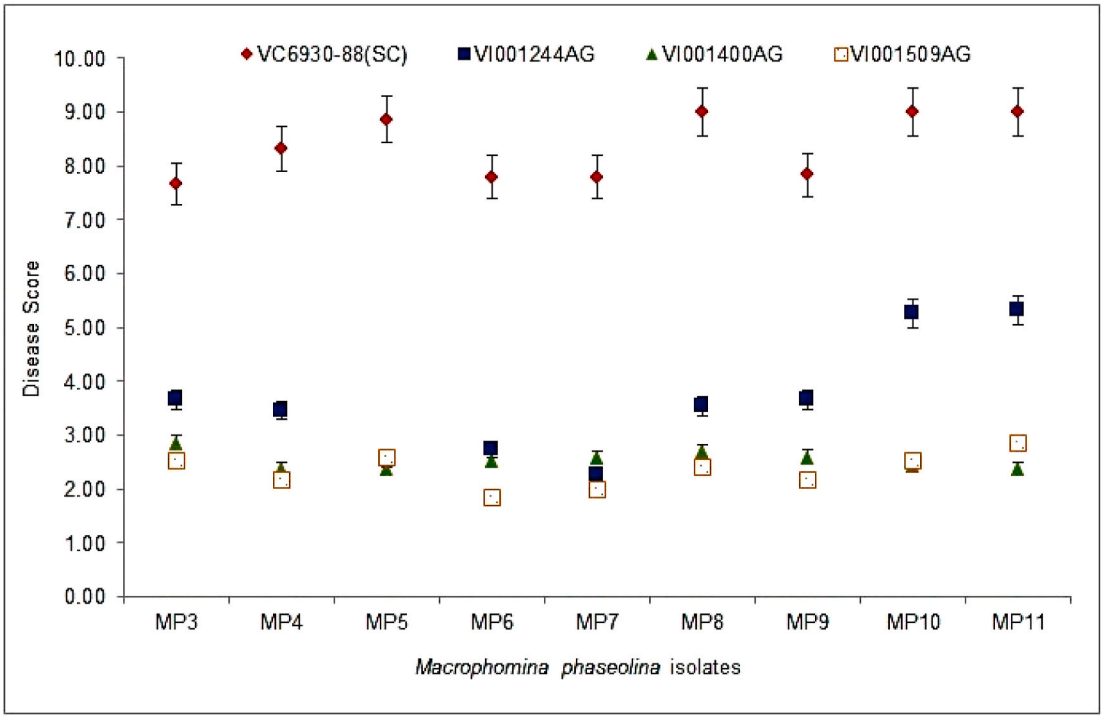

Fig. 6. Disease reactions of identified stable resistant mungbean accessions from glasshouse and field experiments against different MP isolates from India. Disease score was rated on a 1-to-9 scale, where 1 = Immune, 3 = Resistant, 9 = Highly susceptible (For abbreviation of MP isolates please refer Table 1).

for DRR resistance across the four environments. Besides, some accessions, such as VI000766BG VI001268BG VI001282AG, and VI001535BG that were resistant in the sick pots, were found susceptible in the field. The differential reaction of mungbean accessions in sick pots and field may be attributed to the variations in the pathogen's virulence and prevailing environmental conditions (Kumari and Ghatak, 2018). Variability in virulence genes in the pathogen and their subsequent varied responses under different geographical locations may be responsible for varied DRR incidence (Kulkarni and Chopra, 1982).

These three resistant accessions were re-evaluative by the paper towel method against nine isolates of MP from India. Accessions VI001509AG and VI001400AG were resistant against all nine MP isolates, while VI001244AG was resistant against three of them. Therefore, this investigation offers an understanding of the pathogen originated from the diverse locations for the evaluation of mungbean accessions against DRR. The accessions VI001244AG, VI001509AG, and VI001400AG had the lowest levels of DRR incidence and would be the preferred accessions for the breeding programs.

Over the past years, investigations were carried out to evaluate the mungbean accessions for DRR resistance. From Pakistan, accessions NCM 252-10 and 40536 were reported as resistant through the paper towel (Khan and Shuaib, 2007), MNUYT-317 and NM-2011 through the sick pot (Khan et al., 2016), and Azri 2006 has shown DRR resistance under the field conditions (Haseeb et al., 2013). In India, accession 11160a (Dreshka et al., 1974) through the paper towel and KM 4–44, MSJ 118, and KM 4–59 in the field (Choudhary et al., 2011) have shown potential DRR resistance. Our earlier study revealed that IPM-99125, EC693368, and EC693369 accessions showed DRR resistance through the paper towel method, and IPM-99125 had higher plant survival through the sick pot method (Pandey et al., 2020). In the present investigation, accessions VI001244AG, VI001509AG, and VI001400AG were resistant in both glasshouse and field, and also against few MP isolates.

In the breeding program, multi-environment evaluation of accessions is useful for the selection of stable and resistant accessions. The variability in disease reaction response of few accessions in Hyderabad and Yezin reflected the influence of environment/pathogen toward variability of DRR incidence. This study identified durable DRR-resistant mungbean accessions for future resistance breeding programs. The DRR resistance found in accessions VI001244AG, VI001509AG, and VI001400AG will be useful after the identification of quantitative trait loci (QTL) associated with resistance. This can be mapped in the genome of mungbean with markers; enabling marker-assisted selection (MAS) for DRR resistance, as to date no QTL controlling resistance to DRR pathogen in mungbean is reported. Therefore, further investigations are required to develop mapping populations from these DRR resistance sources by crossing them with mungbean breeding accessions to identify the QTLs controlling resistance found in them.

5. Conclusion

In conclusion, the present investigation revealed that mungbean accessions such as VI001244AG, VI001509AG, and VI001400AG demonstrated stable DRR resistance response in paper towel assay, glasshouse assay, and under field conditions. These three accessions also showed a wide-spectrum resistance response against different isolates of MP from India. Therefore, the cultivation of these accessions in DRR disease-prone areas could be recommended for the production of mungbean. The identified resistant accessions could also be deployed as resistant donors for developing the resistant mungbean varieties.

Author contributions

AKP-Conceived and planned the work with significant inputs from RN. AKP-conducted the screening trials in Hyderabad, drafted the manuscript. MY, MMW and HMML- Conducted screening trials in Yezin, Myanmar, data collection and pathogen identification, also in manuscript editing. ZS-Conducted molecular characterization of *M. phaseolina* isolate from Myanmar. AR and AGK- Contributed in the experimental designs, statistical analysis of the data, and in manuscript editing. RN-Finally edited the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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