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Cercospora Leaf Spot (CLS) Disease Resistance Screening of Mung bean [*Vigna radiata* (L.) Wilczek] Germplasms

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ABSTRACT: Mung bean productivity is very sensitive to CLS disease, which needs to be addressed by developing resistance genotypes. During the *pre rabi* seasons of the year 2018 and 2019, a total of ninety different mung bean germplasm accessions were tested in the field condition to determine their level of resistance to the leaf spot disease caused by the fungus *Cercospora canescens*. For the purpose of determining the level of resistance exhibited by the mung bean accessions, a disease rating scale ranging from 1 to 5 was used. In terms of disease reaction, a significant variation among the genotypes was observed. It was found that thirty-two accessions had resistance reaction, and thirty-five accessions had moderately resistance reactions to the CLS disease. According to the findings of the current research, resistant and moderately resistant genotypes that have been identified against the CLS disease, could have the potential to be utilised in the breeding programme, that is being conducted in order to develop mung bean varieties resistant to *Cercospora* leaf spot.

Keywords: *Cercospora* leaf spot, *Cercospora canescens*, Mung bean, Screening.

INTRODUCTION

The mung bean [*Vigna radiata* L. Wilczek], is a grain legume crop of the genus *Vigna* that is widely cultivated for commercial purposes. The quantity of mung bean production in South and Southeast Asia is rising steadily (Priyadarshini *et al.,* 2020). It has a relatively short life cycle and rapid growth when it is being cultivated, both of which contribute to its widespread use (Jat *et al.,* 2012). In addition, if it has a symbiotic interaction with *Rhizobium*, it can fix nitrogen from the atmosphere. This enhances the health of the soil and results in increased agricultural production (Sahoo & Sharma 2018). In comparison to other cereals and legumes, the mung bean does not present the same level of risk to one's health due to its good digestibility, high Vitamin B content, and high protein content. The genome of the mung bean is small, measuring 494-579 mega base pairs (Chand *et al.,* 2015).

Historically speaking, the majority of its cultivation takes place in the Asian region; but, in more recent times, its cultivation has spread to both Africa and the America. However, the yield of mung beans is extremely vulnerable to biotic stresses such as *Cercospora* leaf spot (Sahoo *et al.*, 2021a). Mung bean is prone to the spread of *Cercospora canescens*, the fungus that is responsible for the *Cercospora* leaf spot (CLS) disease (Sahoo *et al.,* 2020a; Sandhu *et al.,* 2022). This necessitates special attention, because the disease can inhibit plant growth and lead to a decrease in seed production. The genetic diversity of *Cercospora* spp. makes it possible for CLS to manifest as a severe disease in a variety of hosts and geographic locations (Joshi, 2006; Das *et al.,* 2020). The *Cercospora* fungus requires infected seed and waste plant material in order to thrive.

CLS is common in Asia, and it causes the most damage in regions that have high relative humidity (79–85%) and daily temperatures that average 22.5–23.5°C (Batzer *et al.,* 2022). The humidity plays a significant role in the germination of conidia (Kumar *et al.,* 2011). The symptoms of the disease often manifest one to two weeks after the plant is inoculated with the pathogen. The pathogens can infect plants at any stage of growth (Samal *et al.,* 2021). The disease becomes more severe as the plant ages, and it is also possible for the diseases to infect the pods during maturity (Bhat *et al.,* 2014; Sahoo *et al.,* 2018; Sahoo *et al.,* 2019). As a result, it is essential to identify CLS disease-resistant cultivars in mung bean and to create a management package that will both minimise the cost of production and protect the environment, during the mung bean cultivation.

MATERIALS AND METHODS

Plant Materials. Ninety mung bean genotypes, including four check varieties, are collected from the Department of Agricultural Biotechnology, College of Agriculture, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar, India and included for screening against CLS resistance in the present study.

Fungal isolation, culture and preparation of inoculums. *Cercospora* infected diseased leaf samples were collected from the Centre for Pulses Research (CPR), Berhampur, India, from the infected cultivar, IPM-02-14 with GPS information NL 190 21' 38'' and EL 840 45' 54'', that displayed the typical CLS symptoms to isolate the pathogen *Cercospora canescens* (Chupp, 1953). After spreading the pathogen spores over a 2% water-agar plate and placing the plates in an incubator at 25°C, the pathogen spores were collected from the ash grey centre of the lesions at the tip of the inoculation needle. Under a microscope (100x), sporulation were observed and marked after an incubation period of six hours. Individual germinated spores were collected using a cork borer that had been cleaned with water agar.

These spores were then deposited on tilted potato dextrose agar in culture tubes. The recognisable characteristics of *Cercospora canescens* included its typical growth, the generation of *cercosporin* (Daub, 1982), and typical conidia and conidiophores (Ellis and Martin, 1882). A pure culture of the isolate was kept at a temperature of 25°C on a medium consisting of potato dextrose agar (PDA). After that, Sorghum grains were boiled until soft without breaking the seed coat in order to facilitate the growth of inoculum. After boiling, the grains were spread out in the shade in order to reduce the amount of excess moisture. Sorghum grains weighing 200 g were placed in a polypropylene bag with dimensions of 30 centimetres by 20 centimetres before being sterilised in an autoclave for 45 minutes at a temperature of 121.6°C and a pressure of 15 pounds per square inch.

After being injected with 10 pieces of fungal mycelia with a diameter of 7 mm collected from a culture that was 15 days old, these bags were kept in an incubator at a temperature of 25°C for 20 days. Mycelia from the fungus *Cercospora canescens* invaded all of the grains. After the bags had been completely colonised, they were aggressively shaken to break up the network of mycelia, and then they were re-incubated at 25 °C. After incubation for 25 days, spore formation was detected (Chand *et al.,* 2013). The inoculum was made by taking grains that had already been colonised and dispersing them in one litre of sterile water. After the sporulated grains were mixed, they were vigorously agitated for 15 minutes in order to detach the spores, and then they were filtered through muslin cloth. In order to get an acceptable level of spore load for inoculation, further dilution of the spore suspension was performed.

Experimental details and screening methodology. CLS disease reaction of a set of ninety genotypes along with suitable susceptible checks such as HUM-8 (Singh *et al.,* 2017), PM-1522 (Singh *et al.,* 2021) and KAMDEV (local check), were evaluated under natural field conditions in the experimental field of Experimental Field station, EB-2 at College of Agriculture, OUAT, Bhubaneswar, during *pre rabi* 2018 and *pre rabi* 2019. Each plot contains three rows of one-meter length and the seeds are sown in a spacing of 30 × 10 cm. As the initial or basal dose of fertilizer, all of the FYM, phosphorous, and potash as well as half of the nitrogen fertilizers were applied. The remaining half of the nitrogen fertilizer was applied 21 days after the seeds were sown. During the process of top dressing, hoeing and hand weeding were also performed. It was ensured that maximum CLS disease pressure would occur by taking all of the essential precautions, such as preserving the ideal level of humidity and planting susceptible checks all along the borders and after every twenty test genotypes. The prepared spore suspension was artificially inoculated to all of the genotypes at 20, 25, and 30 days after planting (DAP), by using a sprayer.

After the crop was inoculated, it underwent consistent checking to determine whether or not the CLS pathogen was present and whether or not the disease was progressing in natural field condition (Ahmad *et al.,* 2013), by monitoring the symptoms including the individual, circular spots that are tan to light brown with reddish purple borders (Fig. 1). After that, a conidium sample was collected from a infected plant, and it was analysed under a light microscope to determine whether or not the symptoms of the disease were caused by an infection with *Cercospora canescens*. Infection on the leaves of each plant was then scored for CLS reaction at 40 DAP on a rating scale of 1–5 (Chankaew *et al.,* 2011), where, 1: resistance (R); 2: moderately resistance (MR); 3: moderately susceptible (MS); 4: susceptible (S), and 5: highly susceptible (HS).

Fig. 1. *Cercospora* leaf spot disease symptoms in mung bean.

RESULTS

The overall CLS reaction under field during the year *pre rabi* 2018 and *pre rabi* 2019 showed occurrence of 35% resistance accessions, 39% moderately resistance accessions, 9% moderately susceptible accessions, 10% susceptible accessions and 7% highly susceptible accessions respectively (Fig. 2). Thirty-two accessions were found resistant, and thirty-five accessions were found moderately resistant reaction against the CLS disease (Table 1). Rest of the accessions were showing either susceptible or moderately susceptible or highly susceptible CLS reaction.

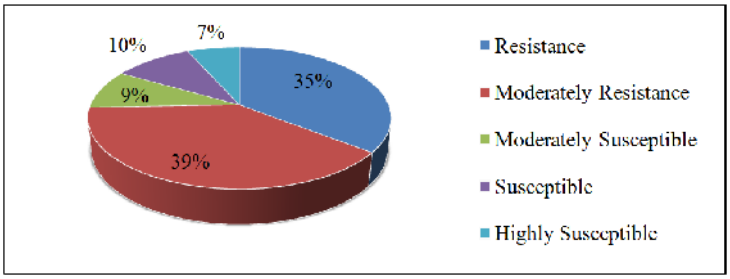

Fig. 2. Distribution of *Cercospora* leaf spot disease reaction across the mung bean genotypes

Table 1: Genotype showed different response against CLS disease of mung bean under field during the year *pre rabi* 2018 and *pre rabi* 2019.

Score	Disease Reaction	No. of genotypes	Name of the genotypes
1	Resistance (R)	32	AVMU-1687, AVMU-1683, AVMU-1698, AVMU-1684, AVMU-1699, BM-212-9, VC-6372, NVL-722, IPM-205-7, IPM-409-4, JHAIN-MUNG-GREEN, RMG-62, AVMU-1691, PM-14-11, PUSA-672, VC-6368-46-40-1, PANT MUNG-8, VGG-16-036, MDGVV-18, ML-2479, RMG-1087, VC-3960-88, VGG-15-29, KNM-1502, HUM-2, BANSA-PAL-GR, RMB-12-07, SML – 1901, NBPGR – 150, CPR BAM GP -289, TARM – 2, COGG-13-39
2	Moderately resistance (MR)	35	NDMK-15-513, EC-693367, PAU-911, PMB-134, AVMU-1682, ML-818, AVMU-1678, SML-1815, KPS-2, IPM-312-9, LM-1907, IPM-410-3, RMG-1092, ASKA MUNG-BL, NARENDRA MUNG-1, MH-13-23, SVM-6133, LGG-460, COGG-13-19, IGKM-6-26-5, EC-693369, VGG-15-30, NBPGR-150, PDM-11, V-1000319-AG, NMK-15-12, PUSA-9072, RMG-364, PUSA-1672, CPR BAM GP -349, MH – 421, PUSA – M – 1771, RMB – 12 – 07, AVMU-1681, IPM-409-4
3	Moderately susceptible (MS)	8	AVMU-1680, GANGA-1, AVMU-16100, KPS-1, SML-1808, PUSA-1841, PUSA – 105, BGS-9
4	Susceptible (S)	9	PUSA-0672,PM-1522,KM-2355, NVL-855, DGG-8, IPM-02-3 , KAMDEV, OBGG-52 , PANT MUNG - 2
5	Highly susceptible (HS)	6	ASHA MUNG, PUSA-BM-1, HUM-8, IPM-312-20, KVK PURI – 4, IPM-02-14

DISCUSSION

Mung bean is a popular pulse crop grown in Pakistan, India, and many other countries. *Cercospora* leaf spot is a major mung bean fungal disease that causes significant yield loss due to poor cultural practises and the incorrect crop rotation system (Marappa, 2008). Fungicides and botanicals can be used to manage the disease, but the most appropriate recommendation is to use resistant mung bean varieties (Marappa, 2008). As a result, the current study's goal was to screen mung bean genotypes for resistance to CLS. The current study's screening results using 90 genotypes revealed a wide variation towards CLS reaction, with scores ranging from 1 to 5. For CLS stress resistance in the studied population, five phenotypic groups were identified: R (resistance), MR (moderately resistance), MS (moderately susceptible), S (susceptible), and HS (highly susceptible). The current study observed thirty-two resistance and thirty-five moderately resistance genotypes.

Previously, researchers confirmed the presence of genetic variation for CLS resistance in mung bean. Different genotypes of CLS disease resistance have been reported from various locations in India. CLS resistance has been discovered in ML-5, ML-15, and ML-3 (Mew *et al.*, 1975). Following that, four genotypes, ML-231, ML-5, ML-267, and ML-337, were resistant to CLS and had high seed yield (Marappa, 2008). In another study, five varieties, CO-4, CO-5, ML-515, BM-4, and TM-98-50, were found to be resistant to CLS, while nine genotypes, LM-1, LM-319, LNM-729, HUM-6, SG-1, AAU-34, TM-98-37, V-461, and VC-3944, were found to be moderately resistant (IIPR Annual report, 2017-18). Similarly, in previous studies, resistant, moderately resistant, and highly resistant mung bean varieties were screened for

CLS resistance. Resistance to CLS was found in 4 genotypes (Gupta *et al.*, 2007), 15 genotypes (Kaur *et al.*, 2011), 10 genotypes (Singh *et al.*, 2004), 4 genotypes (Marappa, 2008), 4 genotypes (Yadav *et al.*, 2014), 8 genotypes (Singh and Singh 2014), and 5 genotypes (Zhimo *et al.*, 2013) of mung bean studied in India. Similarly, according to a previous study, 7 CLS resistance genotypes were obtained in Taiwan (Hartman *et al.*, 1993).

The majority of the mung bean varieties in the present study were found to be resistant or moderately resistant to CLS stress in the current study. It can be concluded that the panel population studied possesses significant genetic variation for CLS resistance (Sahoo *et al.*, 2022). However, agronomically significant variations in crop plants, such as resistance to biotic stresses in mung bean, are controlled by polygenic inheritance of complex traits known as QTLs (Quantitative Trait Loci), which are dependent on the interaction of genetic and environmental factors (Sohail and Fakharuddin, 2021; Nagalla *et al.*, 2022). Mapping and characterising these genomic regions in the mung bean genome using resistance varieties can facilitate marker-assisted breeding for crop improvement towards CLS resistance in mung bean.

CONCLUSION

Based on the current findings, the thirty-two resistance accessions and thirty-five moderately resistant accessions for CLS disease may be used in a breeding programme aimed at developing a high-level resistant mung bean variety against CLS. The findings of this study could also serve as a prerequisite and starting point for launching a crop improvement programme aimed at introducing CLS resistant genes or QTLs into elite mung bean cultivars. The resistance and moderately resistant varieties identified in this study may also be validated by further field screening and then could be used as resistant sources for the introgression of CLS resistant genes into elite mung bean cultivars through advanced molecular breeding approaches.

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Conflict of Interest. None.

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