Research Article

Screening of Mung bean Germplasm for Resistance against Cercospora Leaf Spot Under Natural Field Conditions

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ABSTRACT
Mung bean is an excellent protein source pulse crop grown throughout the world. Punjab province accounts for 80% area and production of mung bean in Pakistan. Mung bean is cultivated on a large scale for its green manuring ability and grain consumption. Mungbean is prone to economically important fungal, bacterial, and viral diseases. Cercospora leaf spot (CLS) caused by Cercospora canescens is one of the most serious disease concerns in Pakistan. The pulses program at the National Agriculture Research Centre, Islamabad is trying hard to get the resistant germplasm against CLS. Sixty-eight advance lines/approved varieties were evaluated in the augmented design under natural field conditions. Forty-one advance lines/approved varieties were found resistant and twenty-five advance lines/approved varieties were found moderately resistant. Two advanced lines/approved varieties did not perform well and were susceptible to CLS infection. This data indicates that the germplasm at pulses program is excellent in resistance against CLS under natural field conditions in the Arid Zone and can serve as potential future varieties.

Keywords: Mung bean, Cercospora leaf spot, Resistance germplasm, Pulses, Screening

INTRODUCTION
Legumes are important source of protein and mung bean (*Vigna radiata* L. Wilczek) is a widely used, nutritionally rich, highly digestible, protein and vitamin source leguminous crop [1,2]. Mung bean is famous with multiple names like green gram, Oregon pea, golden gram, Chickasaw pea, and suey bean and is one of the most important species in the genus *Vigna* [3]. Mung bean grows best in warm-humid climatic conditions with a temperature range of 25°C-35°C [4]. The global area under mung bean production is about 7.3 million hectares which results in about 5.3 million tons produce worldwide [5]. The area is increasing steadily in South Asia which shows interest in the cultivation of mung bean in these areas. Mung bean is cultivated on 18% of the total area of Pakistan devoted for pulses and it yields about 16% in total pulses

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production [6]. The short life cycle of the crop, rapid growth and its nitrogen fixing ability is convincing the farmers to grow more mung bean [7]. In India, successful integration of mung bean is practiced in the maize-wheat system which developed a better income support for Indian farmers and should be considered for Pakistani farmers [8].

Mung bean is susceptible to several pests and pathogens including fungus, bacterial and viral diseases [9, 10]. Although the area is increasing but the insects and pathogen as mentioned above are limiting the yield. Cersopora leaf spot (CLS) is one of the most damaging diseases causing severe economic losses and is caused by *Cercospora canescens* [11, 7, 12]. CLS inhibits plant growth by causing severe leaf spots and ultimately reducing the yield [13]. It usually thrives in infected seed or waste plant material, i.e. debris, etc. The disease is very much prevalent in Pakistan as is the case for other Asian countries and the maximum damage is reported in high humidity areas (79-85%) [3]. CLS in mung bean is widespread in India and 47% yield losses were reported in the humid and warm regions of northeast plains of India [14]. Similarly, 61% yield losses were reported in both Australia and Pakistan which is very serious concern for the mung bean growers [15].

Humidity helps the conidia to germinate, and the symptoms develop within two weeks as the pathogen strikes the crop [16]. Mung bean is susceptible to CLS attack throughout the season, but the early attack will result in severe economic loss [17]. In case of severe attack, pods get infected and serve as a source of inoculum for next season crop [7]. This make the CLS as the most important limiting factor for the mung bean and it needs to be managed with full zeal and zest. CLS resistance in beans is not easy and has not been developed in neighboring countries as information for the desirable traits is insufficient [18]. Some of the information is provided recently like biotic stresses in mung bean is controlled by polygenic inheritance of complex traits which depends genetic and environmental factors interaction [19]. Overall, disease resistance is the key to manage the diseases and keeping this mind the study was planned with the objective of screening of available germplasm at Pulses Program, Crop Science Institute (CSI), National Agricultural Research Center (NARC), Pakistan.

MATERIALS AND METHODS

The pulses program research field area at CSI, NARC was selected to conduct the experiment for the evaluation of 68 mung bean advance lines and approved varieties during the highly humid kharif season of 2023. Lines/approved varieties were labelled as MgAT-1, MgAT-2, MgAT-3, MgAT-4, MgAT-5, MgAT-6, MgAT-7, MgAT-8, MgAT-9, MgAT-10, MgAT-11, MgAT-12, MgAT-13, MgAT-14, MgAT-15, MgAT-16, MgAT-17, MgAYT-1, MgAYT-2, MgAYT-3, MgAYT-4, MgAYT-5, MgAYT-6, MgAYT-7, MgAYT-8, MgAYT-9, MgAYT-10, MgAYT-11, MgAYT-12, MgAYT-13, MgAYT-14, MgAYT-15, MgAYT-16, MgAYT-17, MgAYT-18, MgAYT-19, MgAYT-20, MgAYT-21, MgAYT-22, MgAYT-23, MgAYT-24, MgAYT-25, MgAYT-26, MgAYT-27, MgAYT-28, MgAYT-29, MgAYT-30, MgAYT-31, MgAYT-32, MgAYT-33, MgAYT-34, MgAYT-35, NM-16, AZRI-21, DERA MUNG, NCM-11, E4, NCM-11-8, CHKWAL-MUNG, E2, NGM, NCM-11-22, AZRI-006, NM-06, ABBAS MUNG, MSPS-119, NCM-13, and NM-11(spreader) (Fig. 1). Seed sowing was done by the manual seed drill at 30 cm row to row and 10 cm plant to plant distance. Standard agronomic practices were followed throughout the cropping season. The trial was conducted in augmented design. NM-11 was used as a spreader/susceptible check variety.

Standard use of fertilizers was practiced. Phosphorus, potash, and half of the nitrogen fertilizers were added at the time of sowing. The remaining half bag of urea was applied after 21 days of sowing. Hoeing and hand weeding was performed as and when required during the whole crop season. CLS disease pressure was maximized by taking all the essential precautions like maintaining the ideal humidity, planting susceptible check after every five lines/genotypes, and all along the borders of the experimental unit. Consistent observations were made to note the symptoms of CLS and its progression in the natural field conditions. The individual small circular spots ranging from tan to light brown color symptoms were focused during the study. Samples were collected from the infected plants of the screened lines and grown on potato dextrose agar (PDA) for culture growth and were analyzed under light microscope. Conidium were observed for confirmation of the CLS attack. Scoring for screening of the CLS reaction was performed from leaves of each plant after 60 days of sowing using the Mayye and Datar 0-9 rating scale with some modifications given in Table 1.

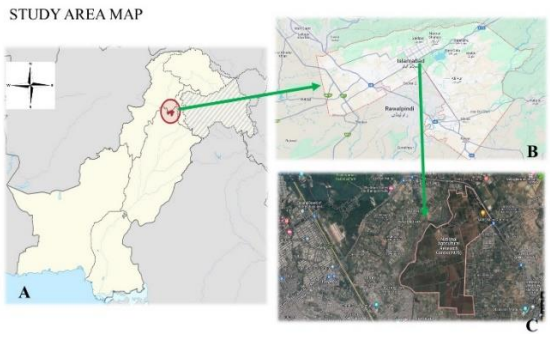

Figure 1. Study area map for the disease screening experiment. A) Pakistan map indicating Islamabad in red. B) Map of Islamabad city showing the area for experimental unit created by the google map. C) Map of National Agricultural Research Center where the study was conducted.

Table 1: Disease severity scale for rating of Cercospora leaf spot

Disease Scale	Percent Infection	Category	Disease Reaction Group
0	No visible symptoms on plants	Immune	I
1	1-10% foliage or pod area affected with small pinhead lesions	Highly Resistant	HR
3	11-20% foliage or pod area affected with small round brown spots	Resistant	R
5	21-30% foliage or pod area affected with large spots	Moderately Resistant	MR
7	31-50% foliage or pod area affected with bigger coalescing spots	Susceptible	S
9	51-100% foliage or pod area affected bigger coalescing spots	Highly Susceptible	HS

RESULTS

Sixty-eight mung bean advance lines or germplasms and approved varieties started germination within a week after sowing and the first symptoms were observed after four weeks in some lines and in some lines, symptoms appear after five weeks. Small circular spots ranging from tan to light brown color were observed during screening (Fig. 2) and conidia observations from the PDA growth under microscope confirmed that the symptoms were caused by *Cercospora canescens*. The percentage of infection ranging from susceptible to resistant reactions observed during CLS screening are given in Fig. 3.

Figure 2. Symptoms observed during the CLS screening. A) Small individual circular spots observed on line number MgAYT-19 during screening. B) Highly infected leaves of NM-11 (Spreader) due to CLS attack during screening.

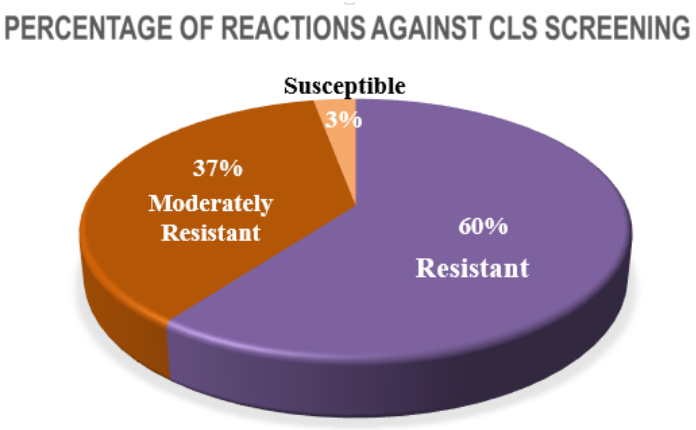

Figure 3. Percentage of susceptible to resistant reactions observed during CLS screening.

On the base of disease rating scale score forty-one lines (60%) were found resistant with 11 to 20% infection on their leaves till maturity (Table 2). Twenty-five (37%) were found to be moderately resistant based on disease rating scale in which infection was 21-30 % (Table 2). Only two lines (3%), namely NM-11 and MgAT-1 were found susceptible based on disease score (Table 2). Individual score for each line is given in Table 3. This data indicated that the germplasm in the breeding process is very clean and bear good resistance against CLS.

Table 2. Response of advance lines/germplasm screened against CLS

Score	Disease Reaction	No. of genotypes	Name of the genotypes
0	Immune	0	-
1	Highly Resistant	0	-
3	Resistant	41	MgAT-1, MgAT-5, MgAT-6, MgAT-7, MgAT-9, MgAT-10, MgAT-11, MgAT-12, MgAT-15, MgAT-16, MgAYT-2, MgAYT-3, MgAYT-4, MgAYT-5, MgAYT-8, MgAYT-9, MgAYT-10, MgAYT-11, MgAYT-12, MgAYT-16, MgAYT-17, MgAYT-18, MgAYT-20, MgAYT-21, MgAYT-26, MgAYT-27, MgAYT-28, MgAYT-30, MgAYT-31, MgAYT-32, MgAYT-33, NM-16, E4, NCM-11-8, CHKWAL MUNG, DERA MUNG, NCM-11-22, AZRI-006, NM-06, ABBAS MUNG, MSPS-119
5	Moderately Resistant	25	MgAT-2, MgAT-3, MgAT-4, MgAT-8, MgAT-13, MgAT-14, MgAT-17, MgAYT6, MgAYT7, MgAYT-13, MgAYT-14, MgAYT-15, MgAYT-18, MgAYT-19, MgAYT-22, MgAYT-23, MgAYT-24, MgAYT-25, MgAYT-29, MgAYT-34, MgAYT-35, AZRI-21, E2, NGM, NCM-13
7	Susceptible	2	NM-11, MgAYT-1
9	Highly Susceptible	0	

Table 3: Response of individual lines/germplasm screened against CLS

Sr. No.	Entry	CLS Score	Response
1	MgAT-1	3	R
2	MgAT-2	5	MS
3	MgAT-3	5	MS
4	MgAT-4	5	MS
5	MgAT-5	3	R
6	MgAT-6	3	R
7	MgAT-7	3	R
8	MgAT-8	5	R
9	MgAT-9	3	R
10	MgAT-10	3	R
11	MgAT-11	3	R
12	MgAT-12	3	R
13	MgAT-13	5	MS
14	MgAT-14	5	MS
15	MgAT-15	3	R
16	MgAT-16	3	R
17	MgAT-17	5	MS
18	MgAYT-1	7	S
19	MgAYT-2	3	R
20	MgAYT-3	3	R
21	MgAYT-4	3	R
22	MgAYT-5	3	R
23	MgAYT-6	5	MS
24	MgAYT-7	5	MS
25	MgAYT-8	3	R
26	MgAYT-9	3	R
27	MgAYT-10	3	R
28	MgAYT-11	3	R
29	MgAYT-12	3	R
30	MgAYT-13	5	MS
31	MgAYT-14	5	MS
32	MgAYT-15	5	MS
33	MgAYT-16	3	R
34	MgAYT-17	3	R
35	MgAYT-18	5	MS
36	MgAYT-19	5	MS
37	MgAYT-20	3	R
38	MgAYT-21	3	R
39	MgAYT-22	5	MS
40	MgAYT-23	5	MS
41	MgAYT-24	5	MS
42	MgAYT-25	5	MS
43	MgAYT-26	3	R
44	MgAYT-27	3	R
45	MgAYT-28	3	R
46	MgAYT-29	5	MS
47	MgAYT-30	3	R
48	MgAYT-31	3	R
49	MgAYT-32	3	R
50	MgAYT-33	3	R
51	MgAYT-34	5	MS

52	MgAYT-35	5	MS
53	NM-16	3	R
54	AZRI-21	5	MS
55	DERA MUNG	3	R
56	NCM-11	5	MS
57	E4	3	R
58	NCM-11-8	3	R
59	CHKWAL MUNG	3	R
60	E2	5	MS
61	NGM	5	MS
62	NCM-11-22	3	R
63	AZRI-006	3	R
64	NM-06	3	R
65	ABBAS MUNG	3	R
66	MSPS-119	3	R
67	NCM-13	5	MS
Spreader	NM-11	7	S

DISCUSSION

Genetic variation and resistance are major criterion for mung bean crop improvement. Mung bean is a popular pulse crop grown in Pakistan, India, and many other countries. Screening is a traditional way of achieving better resistance. The variation in response of the genotypes to any type of stress (i.e. CLS in this case) is a steadfast standard to find out the best and worst-performing genotypes for the selection of parents for use in future breeding program. The minor variation in response of a genotype indicates that the line is more stable and will endure better in diseased conditions. It means that the line is tolerant or even resistant to the disease. On contrary, if major variation occurs in the response, especially if the change is undesirable, it means the infection impacts the genotype making it susceptible [21].

Cercospora leaf spot (CLS) disease is an important foliar disease in mung bean crops but no highly resistant sources are available for production in tropical and subtropical regions. The disease is caused by the fungus which occurs commonly in wet seasons, i.e. monsoon season in Pakistan [20]. Severe defoliation, less chlorophyll pigmentation, etc. can lead up to 50% seed yield reduction. Poor agronomic practices and the inappropriate crop rotation system aggravate the disease. The above-mentioned factors make resistance to CLS highly imperative [21]. Like plant resistance, pathogenic variability is another factor making screening important and pathogenic variability is causing more complications like correct diagnosis of the causal organism. Another factor which makes CLS screening imperative is wide host range of the *Cercospora canescens* [20].

Screening was performed to know the actual status of resistance or susceptibility in the existing germplasm of the country. Mung bean genotypes portrayed outstanding resistance response towards CLS severity and most of the lines showed tolerance/resistance towards this disease in this study. Just like the already reported documents, the disease symptoms commenced on the lower side of the older leaves of susceptible genotypes and gradually spread all over the plant [20]. A substantial variation was observed amongst the 68 subjected lines like NM-11 & MgAYT-1 were susceptible while about 41 lines like MgAT-1 & MSPS-119 were found resistance. Disease score of the genotypes ranged from "3-7". Identification of mung bean lines/genotypes/germplasm/approved varieties with less CLS severity is apposite for accommodating these resistant sources in the existing and future breeding program and integrated disease management [20].

Single recessive gene has been reported for resistance against the CLS which signifies that single gene transfer methods, such as, backcross breeding, can be valuable to incorporate disease resistance into high-yielding mung bean genotypes [22]. In future, the disease screening should be evaluated for the molecular gene analysis which will indicate the responsible genes for resistance.

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