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Identification of resistant sources to stem and root rot of cowpea (*Vigna unguiculata* L.) caused by *Macrophomina phaseolina* (Tassi) Goid

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Abstract
Cowpea stem and root rot caused by *Macrophomina phaseolina* (Tassi) Goid. is a major threat to cowpea cultivation. The disease can cause significant yield losses by infecting the seedlings and adult plants. In addition to reducing yields, the disease also weakens cowpea plants' ability to fix nitrogen, which further increases the pathogen's population in the soil. The greatest approach to managing the cowpea root rot is the host plant resistance since it is both cost-effective and environmentally beneficial. In order to identify sources of genetic resistance to stem and root rot incited by *M. phaseolina,* 154 genotypes of cowpea were screened during *rabi* 2021-22 under sick plot condition in the field at MARS, University of Agricultural Sciences, Dharwad. Out of the 154 genotypes evaluated for their response to stem and root rot in cowpea, five genotypes (EC 724876, EC 724787, EC 724930, EC 240744, IC 625969) exhibited a resistant reaction, while 29 genotypes displayed a moderately resistant response. In contrast, 56 genotypes were moderately susceptible to the disease and the remaining 64 genotypes were identified as susceptible to *M. phaseolina*. These genotypes can also be used as a source of resistance in breeding programmes to create root rot-resistant cultivars.

Keywords: Cowpea, stem and root rot, resistant, host plant resistance

1. Introduction

Cowpea (*Vigna unguiculata* L.) is one of the most ancient human food sources and short duration multipurpose pulse crop grown extensively in tropical and subtropical countries. It is also called as vegetable meat due to high amount of protein in grain with better biological value on dry weight basis. Cowpea grain contains 23.40 percent protein, 1.80 percent fat and 60.30 percent carbohydrates and it is rich source of calcium and iron (Alexandre *et al*., 2016) [3]. It is usually the first crop to be harvested before the cereal crops are ready and therefore is referred to as “hungry-season crop”. India is a major producer of cowpea, with an area of 4 million hectares (mha) under cultivation and a production of 2.7 million tonnes (mt). However, productivity is low, at 567 kilograms per hectare (kg/ha). This is due to a number of factors, including both biotic (diseases, pests) and abiotic (moisture, soil fertility) stresses. Diseases play a significant role in reducing cowpea yields in India (Sindushree *et al*., 2023) [18].

Stem and root rot incited by *Macrophomina phaseolina* (Tassi.) Goid. has been rated as most devastating disease of cowpea. The disease causes extensive damage in *rabi* summer season in India. The first symptom of the disease is yellowing of the leaves which droop in next 2 or 3 days and withers off. The plant may wilt within a week after the appearance of first symptom. When stem is examined closely, dark lesion may be seen on the bark at the ground level. If the plants are pulled from soil the basal stem and main root may show dry rot symptoms, the tissues are weakened and break off easily. In advanced cases microsclerotia may be seen scattered on the affected tissues. The fungus invades the host both inter and intracellularly, it grows rather fast covering large areas of the host tissue and eventually killing them in short time. It produces numerous microsclerotia on host tissue, which measure about 110-130 µ in diameter. Often the conidial or pycnidial stage is produced on the host (Nitharwal, 2019) [11].

The fungus is a facultative parasite capable of living saprophytically on dead organic tissue, particularly on many of its natural hosts producing microsclerotia, which produces pycnidia. When atmospheric temperature is above 30 °C and the pycnidiospores remain viable for over a year since the fungus attack wide range of plant species (Avanija *et al*., 2023) [8]. The fungus is mainly a soil dweller and spreads from plant to plant through irrigation water, implements and

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cultural operation. The sclerotia and pycnidiospore may also become air borne and cause further spread of the pathogen (Rangaswami and Mahadevan, 2008) [16]. According to Nair *et al*. (2020) [14], dry root rot results in a 10–44 percent yield loss in the production of mungbean in India and a 33-44 percent yield loss owing to *Rhizoctonia* root rot. In addition to decreasing crop yields, pathogen damage lessens their ability to fix nitrogen in the soil, which raises disease concentrations there (Khaledi *et al*., 2015) [6].

Management of stem and root rot caused by *Macrophomina phaseolina* is challenging due to the pathogen's polyphagous nature and ability to survive in the soil through its resting structures. Fungicides are expensive and harmful to the environment, making them a less desirable control option. Host plant resistance is a more economical and environmentally friendly approach to managing root rot. This study aimed to identify resistant sources of stem and root rot in cowpea genotypes.

2. Materials and Methods

An experiment was conducted at Main Agricultural Research Station (MARS), University of Agricultural Sciences (UAS), Dharwad during *rabi* 2021-22 with an objective to identify promising sources of resistance to stem and root rot of cowpea caused by *M. phaseolina*. Totally 153 cowpea genotypes received from the Department of Genetics and Plant Breeding, college of Agriculture, UAS, Dharwad and Indian Institute of Pulse Research (IIPR), Regional Centre, Dharwad were subjected for screening under sick soil conditions. The pathogenic strain of *Macrophomina phaseolina* was isolated from diseased stems of cowpea, pure culture was done by single hyphal tip method and cultures were maintained on Potato dextrose agar media, was multiplied on the sorghum grains. The grains were first half-boiled in water, dried Over Night and filled in 500 ml Erlenmeyer conical flasks to 1/4th of their capacity and sterilized at 15 lbs pressure at 121 °C for 15 minutes. Thereafter, this *M. phaseolina* discs of 5 mm was inoculated in to the sterilized sorghum grains containing flasks and incubated at 28+2 °C for 15-20 days. The flasks were shaken every day (Choudhary *et al*., 2011) [4].

Artificial inoculation was made by soil application of giant culture of *Macrophomina phaseolina*. Before sowing all the seed furrows was uniformly applied with mass multiplied inoculum of *M. phaseolina* and the cowpea genotypes were sown with spacing of 45 cm × 20 cm. The disease incidence was recorded using 0-4 scale. Based on percent of incidence genotypes was categorized as immune, resistant, moderately resistant, moderately susceptible and susceptible. The percent disease incidence was recorded and the reaction was categorized according to disease scale given by Nitharwal (2019) [11] with slight modifications (Table 1). Data was analysed through Online Statistical Analysis Tools (IBM SPSS Statistics).

2.1 Percent disease incidence

Percent disease incidence (PDI) was recorded at 30, 60 and 75 DAS by using the formula given by Wheeler (1969) [19].

$$\text{Percent disease incidence (PDI)} = \frac{\text{Number of plants infected}}{\text{Total number of plants observed}} \times 100$$

Table 1: Disease rating scale of stem and root rot of cowpea (Nitharwal, 2019) [11]

Disease scale	Percent incidence	Disease reaction
0	0	Immune
1	0.1-10	Resistant
2	10.1-20	Moderately resistant
3	20.1-30	Moderately susceptible
4	> 30	Susceptible

3. Results and Discussion

Totally 154 genotypes were screened against stem and root rot of cowpea caused by *M. phaseolina* during *rabi* 2021-22 under sick plot condition in the field to identify the resistant sources as described in “Material and Methods” and data are presented in Table 2.

Among the 154 genotypes evaluated, none of the genotypes shown immune or completely resistant reaction but only five genotypes showed resistant reaction with root rot incidence of 0.1 to 10 percent *viz.,* EC 724876, EC 724787, EC 724930, EC 240744, IC 625969, whereas, 29 genotypes showed moderately resistant reaction (EC 725013, IC 402159, IC 608642, EC 725011, EC 107193, IC 606218, EC 101970, IC 39903, EC 3178, EC 3179, IC 202705, IC 202711, IC 202730, IC 201083, IC 202842, IC 198329, IC 202926, IC 215015, IC 198383, IC 198329, EC 724874, EC 3421, PGCP 75, KBC 12, KBC 9, CPD 273, CPD 331, CPD 269, PGCP 6) with root rot incidence of 10.1 to 20 percent.

While, 56 genotypes showed moderately susceptible reaction *viz.,* GC 1801, PCG 4241, DC 18-1, Arka Garima, Pant Lobia 4, GC 1906, ICBC 11, ICBC 9, Pant Lobia 3, KBC 11, ICBC 12, SKAU 6-411, SKAU WCP 149, MFC 09-1, AV 5, PGCP 74, PGCP 73, PGCP 76, PGCP 6, Jowhar, DC 16 (New), IC 402159, IC 345622, EC 738131, IC 402175, EC 240744, IC 34270, EC 103769, IC 420042, IC 91339, IC 91505, EC 240902, IC 613405, EC 390237, EC 724908, EC 724911, EC 40218, EC 3180, IC 202798, IC 202803, IC 97834, IC 202925, IC 91505, IC 257427, IC 259063, IC 202842, IC 20282 11, IC 201087, IC 259073, IC 202786, IC 198355, IC 97829, IC 202709, IC 257427, IC 202799, IC 202833 with root rot incidence of 20.1 to 30 percent.

Remaining 64 genotypes were found susceptible to *M. phaseolina* with root rot incidence of more than 30 percent (IC 259061, IC 201087, IC 202703, IC 202807, IC 606514, EC 724895, EC 725013, IC 393710, EC 101994, EC 725011, IC 4021, EC 402098, IC 402103, IC 203320, EC 725256, IC 402106, EC 72566, EC 724947, IC 606720, IC 4506, EC 784878, IC 605681, IC 202864, EC 724930, IC 214757, IC 97829, IC 367698, IC 202932, IC 202849, EC 309500, EC 390272, EC 39265, EC 340923, EC 390204, EC 367689, EC 332354, EC 309498, IC 202790, IC 215015, IC 202731, IPCP 18-12, PCP 0306-1, GC 1612, GC 1603, SAPG 60, TPTC 29, VCP 1802, NCP 18-013, ICBC 11, DC 15, DC 16, DC 47-1, C 152, TVY 944, KBC 7, UKD 35, KM 5, PKB 6, PKB 4, IT 38956-1, KBC 1, KBC 2, RC 101, GC 3).

In majority of the crops, including black gram (*Vigna mungo*

L.) and several other grain legumes, complete resistance to *M. phaseolina* has not yet been recorded (Sajeena *et al.* 2004, Rao 2008) [17, 15]. Finally, EC 724876, EC 724787, EC 724930, EC 240744 and IC 625969 are the six genotypes which were identified as resistant and these genotypes can be used for crop improvement programmes after further testing.

The results are in agreement with previous reports which demonstrated that, out of twenty-six cowpea germplasm lines screened against dry root rot caused by *Rhizoctonia bataticola* reveals that, no entry was found highly resistance at flowering stage. Two lines *viz.*, PGCP-67, PGCP-64 showed resistant reaction at flowering stage. Fourteen lines have been found moderately resistant *viz.*, DC-15, GC 3, PCP-1131, GC – 1603, PCP-1118, CPD-302, SKAU-C-407, RC 101, MC 17-1, CPD-293, Phule PCP-1123 (AVT-1), Pant Lobia-3, PCP 0306 and VCP 12006. However, ten entries were susceptible at flowering stage and disease intensity was minimum in PGCP-67 and the maximum disease intensity was recorded in VCP 14005 (Koli, 2019) [7].

Researchers have screened different crops for resistance to *M. phaseolina*, including sunflower (Mirza *et al*., 1982) [9], chickpea (Pande *et al*., 2004) [13] and cowpea (Abawi and Pastor-Corrales, 1990) [1]. Similarly, Choudhary *et al*. (2011) [4] tested 25 mungbean genotypes for resistance to dry root rot in the field and found that three genotypes, MSJ-118, KM 4-44, and KM 4-59, were resistant. The findings are in line with Haseeb *et al.* (2013) [5] previous work, in which they evaluated 27 mungbean genotypes for resistance to *M. phaseolina* in the field using artificial inoculation. They discovered that none of the 27 genotypes were completely resistant, but that the genotypes Azari 2006, NM 2006 and AUM-9 were resistant.

Oladimeji *et al*. (2012) [12] screened five cultivars of cowpea against *Macrophomina phaseolina* infection using two methods of inoculation *viz*, pouring of spore or mycelial suspension in the soil and wrapping of inoculums meal around wounded lower stem of the seedling. Cowpea cultivar ITO4K-217-5 was resistant to the pathogen in both inoculation methods. Six cultivars of cowpea were screened against *M. phaseolina* under artificial conditions. None of the variety was found immune or resistant to stem and root rot of cowpea. Among these six varieties RC-19 and Pant lobia-1 were observed moderately resistance, RC-101, Pant lobia-2, Pant lobia-3, Pant lobia-4 were found moderately susceptible (Nitharwal, 2019) [11].

Akhtar and Shoaib (2018) [2] screened 26 genotypes, and reported that 2 genotypes (MNUYT-317 and NM-2011) were highly resistant, and other 10 genotypes were recorded as moderately resistant. Pandey *et al.* (2020) [14] evaluated 43 mungbean genotypes for resistance to *Macrophomina phaseolina* using the rolled paper towel technique. Resistant genotypes with low disease scores were further evaluated using the sick pot technique. IPM99-125 consistently outperformed other genotypes in terms of plant survival.

Most researchers have screened germplasm for resistance to *M. phaseolina* and identified resistant or moderately resistant germplasm during selection. Similarly, in this study, we found resistant and moderately resistant genotypes to *M. phaseolina*, which can be used in crop improvement programs after further testing. These findings are consistent with previous studies.

Table 2: Reaction of cowpea genotypes to stem and root rot under sick plot conditions during *rabi* 2021-22

Reaction	Percent disease incidence	No. of genotypes	Genotypes
Resistant	0.1 - 10	5	EC 724876, EC 724787, EC 724930, EC 240744, IC 625969
Moderately resistant	10.1 - 20	29	EC 725013, IC 402159, IC 608642, EC 725011, EC 107193, IC 606218, EC 101970, IC 39903, EC 3178, EC 3179, IC 202705, IC 202711, IC 202730, IC 201083, IC 202842, IC 198329, IC 202926, IC 215015, IC 198383, IC 198329, EC 724874, EC 3421, PGCP 75, KBC 12, KBC 9, CPD 273, CPD 331, CPD 269, PGCP 6
Moderately susceptible	20.1 - 30	56	GC 1801, PCG 4241, DC 18-1, Arka Garima, Pant Lobia 4, GC 1906, ICBC 11, ICBC 9, Pant Lobia 3, KBC 11, ICBC 12, SKAU 6-411, SKAU WCP 149, MFC 09-1, AV 5, PGCP 74, PGCP 73, PGCP 76, PGCP 6, Jowhar, DC 16 (New), IC 402159, IC 345622, EC 738131, IC 402175, EC 240744, IC 34270, EC 103769, IC 420042, IC 91339, IC 91505, EC 240902, IC 613405, EC 390237, EC 724908, EC 724911, EC 40218, EC 3180, IC 202798, IC 202803, IC 97834, IC 202925, IC 91505, IC 257427, IC 259063, IC 202842, IC 20282 11, IC 201087, IC 259073, IC 202786, IC 198355, IC 97829, IC 202709, IC 257427, IC 202799, IC 202833
Susceptible	>30	64	IC 259061, IC 201087, IC 202703, IC 202807, IC 606514, EC 724895, EC 725013, IC 393710, EC 101994, EC 725011, IC 4021, EC 402098, IC 402103, IC 203320, EC 725256, IC 402106, EC 72566, EC 724947, IC 606720, IC 4506, EC 784878, IC 605681, IC 202864, EC 724930, IC 214757, IC 97829, IC 367698, IC 202932, IC 202849, EC 309500, EC 390272, EC 39265, EC 340923, EC 390204, EC 367689, EC 332354, EC 309498, IC 202790, IC 215015, IC 202731, IPCP 18-12, PCP 0306-1, GC 1612, GC 1603, SAPG 60, TPTC 29, VCP 1802, NCP 18-013, ICBC 11, DC 15, DC 16, DC 47-1, C 152, TVY 944, KBC 7, UKD 35, KM 5, PKB 6, PKB 4, IT 38956-1, KBC 1, KBC 2, RC 101, GC 3

4. Conclusion

Among the 154 genotypes screened against stem and root rot of cowpea during *rabi* 2021-22, five genotypes showed resistant reaction (EC 724876, EC 724787, EC 724930, EC 240744, IC 625969) whereas, 29 genotypes showed moderately resistant reaction. While, 56 genotypes showed moderately susceptible reaction and remaining 64 genotypes were found susceptible to *M. phaseolina*. The resistant genotypes identified in the present study can be utilized as potential donors for future resistance breeding programme against stem and root rot in cowpea.

5. Acknowledgment

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6. Conflict of 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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