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Identification of Stable Resistant Genotypes against Charcoal Rot of Maize (*Zea mays* L.) incited by *Macrophomina phaseolina* (Tassi) Goid

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ABSTRACT: Charcoal rot of Maize caused by *Macrophomina phaseolina* is a major biotic stress responsible for increased yield losses in arid and semi-arid regions especially in areas with moisture stress coincides at flowering stage of the crop. The stalk rot infected plants can be recognized by grayish streaks on stem and numerous black microsclerotia visible on cut opened, shredded vascular bundles with charred appearance. The best way to manage the disease is through identification of charcoal rot resistant sources. Maize Research Centre (MRC), Hyderabad is a nationally identified centre for charcoal rot screening by Indian Institute of Maize Research (IIMR), Ludhiana, India. In the process of maize hybrid development with post flowering stalk rot resistance at MRC, a total of 36 elite inbreds along with resistant and susceptible checks were screened under field conditions continuously by following tooth pic-method of disease screening during *Kharif* 2020, 2021, *Rabi* 2020-21 and 2021-22 and identified six inbreds namely BML-100, BML-101, BML-102, BML-103, BML-106, and BML-108 are identified as lines with stable resistance to charcoal rot of maize with disease score less than 3 on 1-9 scale during all the seasons tested. The lines may be used as resistance sources in breeding for development of new maize genotypes with charcoal rot disease resistance.

Keywords: Charcoal rot, Inbreds, Genotypes, *Macrophomina phaseolina*, Maize.

INTRODUCTION

Maize (*Zea mays* L.) is a most versatile emerging crops having wider adaptability under varied agro-eco-systems. It is in cultivation at tropics, sub tropics and temperate regions under irrigated and rainfed conditions. Maize is not just an important source of human nutrients, but also a primary element of animal feed and raw material for producing many industrial products. The products include corn starch, maltodextrins, corn oil, corn syrup and products of fermentation and distillation industries.

Presently, 1211.64 million tonns of maize is being produced together by over 170 countries from an area of 191.89 million ha with an average productivity of 5.6t/ha (Ann., 2021) Globally. India has the 4th largest acreage and 5th largest corn producer, which means India has 4% area with 2% of global production (India stat, 2021). Currently maize is cultivated in India on an area of 9.47 m ha with production and productivity of 28.7 mt and 3.0 t/ha, respectively (Directorate of Economics & Statistics, DAC&FW). In Telangana state, it is cultivated in 5.43 lakh hectares, of which, 4.26 lakh ha during *Kharif* season and 1.17 lakh ha during *rabi* season (2018-19) with a production of 13.23 lakh tonns and 7.54 lakh tonns. The current productivity level of the State is limited to 3.8 t/ha mainly due to its cultivation as rainfed crop during *Kharif* season.

Maize is affected by different biotic and abiotic stresses. Out of all, the biotic stresses, diseases are one of the major constraints in realizing the potential yields of this crop. The important diseases in India are Post flowering stalk rots, Turcicum leaf blight, Maydis leaf blight, Banded leaf and sheath blight, Brown stripe downy mildew, Polysora rust, Sorghum downy mildew, Rajasthan downy mildew and Bacterial stalk rot. Among all the diseases, charcoal rot (*M. phaseolina*), is dominating in Telangana state. Charcoal rot of maize caused by *Macrophomina phaseolina* (Tassi) Goid *Rhizoctonia bataticola* (Teleomorphic stage) reported as major disease responsible in increased yield losses in arid and semiarid regions especially where moisture stress coincides with flowering stage of the crop like rainfed maize growing areas of Telangana, Karnataka and Tamil Nadu, where the dry spell coincides with flowering stage of maize. Yield losses as high as 70 per cent have been documented. The disease is particularly prevalent in drought years and in arid regions where maize is regularly cultivated in rotation with other host crops. The disease is heat and stress (drought) driven coupled with heavy applications of nitrogen fertilizer. Most of the commercially grown cultivars have shown a high level of disease incidence around grain filling stage. Charcoal rot overwinters or survives as resting structures (microsclerotia) on lower stem residues that remained in the field after harvesting. The yield loses due to charcoal rot (*M. phaseolina*) in India recorded time to time, ranged from 10 to 42% (Desai *et al.*, 1992), 25 to 32% (Kumar,2022), 10.18 to 31.08% (Harlapur *et al.*, 2002). In a field survey conducted during 2019-2020 in Telangana state reported the stalk rot incidence ranged from 27% to 76.8% and yield loss of 30% (Mamatha *et al*., 2020).

The level of disease intensity and extent of damage due to pathogens largely depend on edaphic factors, inoculums density of pathogen sand host plant resistance. Host plant resistance (HPR) is the practical, feasible and effective method to control plant diseases. In spite very few chemicals are used for PFSR management for some extent, these chemicals neither farmer- nor environment-friendly and become serious threat to soil and human health too. Therefore, exploitation of host plant resistance is a first line of IDM and to minimizes inoculums in ecosystem and yield loss in an eco-friendly and economic manner. Over years, germplasm screening at hot spots areas helped in identification of stable sources of resistance, which have been deployed in the development of disease resistant cultivars of maize. Currently very few genotypes are known/available which have stable disease resistance for use in breeding programs, especially in public breeding programs (Krishna *et al* 2019). Kalpana *et al.* (2022 at Udaypur, Rajasthan also identified few maize lines resistance to *Fusarium verticilliodes*. Considering the importance of charcoal rot as major bottleneck in Telangana state for attaining the maximum yield potential in maize as the state is characterized by semiarid nature. Maize Research Centre, ARI, Rajendranagar, PJTSAU is one of the nationally identified hotpot for maize charcoal rot screening program of AICRP on Maize, India.

So, keeping this in view the present work was aimed to identify the stable charcoal rot resistant genotypes which could be used in future for developing promising maize hybrids.

MATERIALS AND METHODS

A total of 35 elite maize inbreds developed at Maize Research Centre, PJTSAU, Hyderabad were used for screening against Charcoal rot of maize under field conditions by artificial inoculation method. The experiment were conducted in during *kharif* 2020, 2021 and *rabi* season 2020-21 &2021-22. The plants were raised in single row and replicated twice with a spacing of 60×20 cm along with susceptible check s Kaveri-50 and resistant check JCY-2-7 in Alfa lattice design. Planted susceptible cultivar K-50 at every20 test rows in the whole field. These rows served as checks, and helped in monitoring the plot. The observations were considered only if susceptible check exhibit more than 90 percent infection. Recommended agronomic practices were followed to establish good crop stand.

Virulent pathogen isolated from maize plants showing typical symptoms, identified and is under use for AICRP on maize screening program was used. Tooth pick inoculation is followed for this disease screening, Payak and Sharma (1983). Round bamboo tooth picks about 6.5 cm long are boiled three times (about1hour each time) in tap water to remove toxic substances. After each boiling these are thoroughly washed in fresh water and dried in the sun. When these are thoroughly dry, they are loosely packed in bundles and put into the glass jars/ bottles and enough potato dextrose broth (one- third length of toothpicks) is added to thoroughly moisten the toothpicks plus some quantity in the bottom of the jars. The jars with the toothpicks are autoclaved immediately after the broth is added. Later the sterilized toothpicks are inoculated with the culture of the pathogen aseptically. The growth of the fungus covered the toothpicks and inoculums was readyforuse10days.

Inoculations were carried at pre flowering stage of maize plants (50-55 DAS). For inoculating plants, the lower internode (second/third) above soil level is opened with a jabber and the tooth pick is inserted into the hole. The jabber is made by driving a nail of the diameter of the toothpick into a wooden handle. The head of the nail is ground off to a point and to the desired length (2cm). The round tooth picks effectively sealed the hole in the stalk. Increased the pathogen load by applying excess nitrogen fertilizer and created moisture stress in field.

Disease scoring. The disease symptoms were scored at 40 days after inoculation by splitting the stalk of inoculated plants. Longitudinally split stalks were individually scored on1-9 rating scale (AICMIP, 1983). The detailed scale was described as follow in (Table 1).

Table 1.

Rating Scale	Intensity and extent of severity (%)	PDI	Disease reaction
1.0	25 per cent of the inoculated internode discoloured	≤11.11	Resistant (R) (Score: ≤ 3.0) (PDI: ≤ 33.33)
2.0	26-50 per cent of the inoculated internode discoloured	22.22	
3.0	51-75 per cent of the inoculated internode discoloured	33.33	
4.0	76-100 per cent of the inoculated internode discoloured	44.44	Moderately resistant (MR) (Score: 3.1–5.0) PDI: 33.34-55.55)
5.0	Discolouration of less than 50 per cent of adjacent internode	55.55	
6.0	Discolouration of more than 50 per cent of adjacent internode	66.66	Moderately suceptible (MS) (Score: 5.1–7.0) (PDI: 55.56-77.77)
7.0	Discolouration of three internodes	77.77	
8.0	Discolouration of four internodes	88.88	HiglySusceptible (S) (Score: ≥ 7.0) (PDI: ≥77.77)
9.0	Discolouration of five or more internodes and premature death of plant	99.99	

Table 2: Screening of maize inbred lines against Charcoal rot of maize caused by *M.phaseolina*.

Sr. No.	Name of the genotype	Disease reaction				
		Kharif, 2020	*Rabi*, 2020-21	*Kharif*, 2021	*Rabi*, 2021-22	Mean
1.	BML-108	3.3	2.9	2.79	2.5	2.9
2.	GP 19	4.2	4.5	3.57	4.0	4.1
3.	GP 16	3.8	3.5	4.0	4.0	3.8
4.	BML 14	3.0	3.5	2.7	3.5	3.2
5.	BML-106	2.5	2.8	3.0	2.3	2.7
6.	BML-105	5.5	5.0	3.7	4.5	4.7
7.	BML-101	2.5	3.0	3.1	2.4	2.8
8.	BML-104	3.7	3.5	3.1	3.5	3.5
9.	BML-107	6.7	4.5	4.4	5.0	5.2
10.	BML-102	2.7	3.3	3.0	2.6	2.9
11.	BML-130	3.5	2.5	2.9	2.6	2.9
12.	PFSR 56	4.0	2.5	4.4	4.0	3.7
13.	PFSR 46	4.3	3.0	5.4	4.0	4.2
14.	BML-103	3.2	2.3	3.0	2.4	2.7
15.	SCGP 74-1	7.2	2.5	6.4	7.0	5.8
16.	SCGP 2	7.2	3.8	6.6	5.0	5.7
17.	SCGP 7	7.3	4.0	8.0	7.0	6.6
18.	SCGP 44-1	5.7	5.5	6.0	5.5	5.7
19.	SCGP 57	3.3	3.5	3.1	3.5	3.4
20.	SCGP 211	3.7	4.5	6.0	4.0	4.6
21.	SCGP 36	6.3	4.5	6.0	5.0	5.5
22.	QPM 46-1	6.5	4.3	3.6	4.0	4.6
23.	QPM 6-10-1	4.7	4.5	6.4	5.0	5.2
24.	QPM 1433	5.3	5.5	3.0	5.5	4.8
25.	PC 9	4.5	4.0	7.0	5.0	5.1
26.	PC 8	4.8	3.0	3.0	4.0	3.7
27.	PC 28-1	4.8	5.5	4.6	5.5	5.1
28.	PC 116	5.3	7.0	6.0	7.0	6.3
29.	MGC 102	4.7	7.5	4.6	5.0	5.5
30.	BML 10	7.8	6.8	5.4	5.0	6.3
31.	MGC 49	6.2	6.3	6.4	6.0	6.2
32.	MGC 157	3.0	2.5	3.0	4.0	3.1
33.	GP 360	6.2	6.0	4.0	5.0	5.3
34.	GP-68	6.5	5.0	6.7	6.0	6.1
35.	GP-47	5.5	6.8	7.0	6.2	6.4
36.	GP-37	6.5	5.5	5.7	5.4	5.8
37.	K-50 (S-Check)	7.5	8.0	7.7	7.8	7.8
38.	JCY-2-7 (R-Check)	3.0	2.8	3.0	2.9	2.9

Table 3: Classification of maize genotypes, screened against *M.phaseolina* charcoal rot into different disease reaction groups.

Sr. No.	Disease reaction	Inbred lines	Disease reaction Range
1.	Resistant (R)	BML-100, BML-101, BML-102, BML-103, BML-106, BML-108 (6)	(Score: $\leq$ 3.0) (PDI: $\leq$ 33.33)
2.	Moderately resistant (MR)	GP 16, GP 19, BML-105, BML-14, PFSR 56, PFSR 46, SCGP 57, SCGP-211, QPM 46-1, QPM 1433, PC 8, MGC-157(12)	(Score: 3.1–5.0) (PDI: 33.34-55.55)
3.	Moderately susceptible (MS)	BML-107, SCGP 74-1, SCGP 2, SCGP 7, SCGP-57, SCGP 44-1, SCGP 36, QPM 6-10-1, PC 9, PC 28-1, PC 116, MGC 102, BML 10, MGC 49, GP 360, GP 68, GP-37, GP 47 (18)	(Score: 5.1–7.0) (PDI: 55.56-77.77)
4.	Highly Susceptible (S)	—	Score: $\geq$ 7.0) (PDI: $\geq$77.77)

Date was recorded on10 plants in each row and mean of the disease score on ten plants is represented as mean score of that genotype for the season. A total of four seasons (two *kharif* and two *Rabi)* was tested and average consistent performance of the disease score is considered to declare resistant/susceptible genotype. The genotypes were grouped in to Resistant, Moderately Resistant, Moderately Susceptible and Highly Susceptible based on the disease reaction.

RESULTS AND DISCUSSION

Identification of novel source of resistance against charcoal rot is very important for developing disease free hybrids which reduce the inoculums load, extends the life of genotype and reduce the cost of cultivation. A total of 35 maize inbreds along with resistant and susceptible checks were screened against *M. phaseolina* under field conditions following toothpick method.

Out of the 35 elite inbred lines screened against *M. phaseolina* during two *kharif 2020, 2021*and two *Rabi* 2020-21, 2021-2022, seasons continuously, noticed the differential behavior of genotypes against charcoal rot pathogen as listed in Table 2&3. Six inbred lines BML-100, BML-101, BML-102, BML-103, BML-106, and BML-108 showed resistant,12 inbred linesGP 16, GP 19, BML-105, BML-14, PFSR 56, PFSR 46, SCGP

57, SCGP-211, QPM 46-1, QPM 1433, PC 8 and MGC-157 moderately and 18 inbred lines BML-107, SCGP 74-1, SCGP 2, SCGP 7, SCGP-57,SCGP 44-1, SCGP 36, QPM 6-10-1, PC 9,PC 28-1, PC 116, MGC 102, BML 10, MGC 49, GP 360, GP 68, GP-37 and GP 4 recorded susceptible reaction. The inbred lines differed significantly for their response against pathogen. Significant variation in resistance was detected in the inbreds evaluated for charcoal rot disease. The similar results reported by Meena *et al.* (2010) who made extensive screening and identified three resistant lines, namely PFSR-13-5, JCY2-2-4-1-1-1-1 and JCY3-7-1-2-1-b-1.

Twenty inbred lines of maize were tested by Kaur *et al*. (2010) in monsoon 2005 and 2006 under artificial epiphytotic conditions for the charcoal rot (*M. phaseolina*). The genotypes evaluated were divided into three groups, resistant, moderately resistant, and susceptible, according on how they responded to the illness. The severity of charcoal rot was measured using a 1–9 scale and identified one genotype, E-10 LET DR99 × Ent 49-2, as resistant line.

Gopala *et al*. (2016) also screened 34 genotypes against *M. phaseolina* and identified only four lines *viz.*, H 37, E 618, 18527 and 18758 as resistant, ten lines *viz*., H 62, 14933, H 109, P 503, P 408, E 684, P 364, E 613, P 345, 18855 as moderately resistant, thirteen lines *viz*., H 61, BML 6, H 10, P 320, H 182, 15026, H 100, H 68, 14982, 18833, 18834, HM 8, PC 4 as susceptible.

Mir *et al*. (2018) screened set of maize inbreds for *F. moniliforme* and *M. phaseolina* under sick plot accompanied by toothpick inoculation method and studied the gene action contributing for stalk rot resistance. They reported that predominant additive gene effect inferring towards resistance to these diseases.

Krishna *et al*. (2019) also screened seventeen lines against charcoal stalk rot of maize in two locations. The results indicated that the disease reaction ranged from 1.3 to 6.5 at Delhi and 2.5 to 7.7 at Ludhiana. Among the inbreds, none of the inbreds were rated as resistant with score below 3.0. However, the inbreds DQL 2008-1, DQL 2009, DQL 2010, DQL 2015, DQL 2028, DQL 2031, DQL 2034, DQL 2039, and DQL2071 were found moderately resistant with score between 3.1 and 6 at both locations.

Kalpana *et al.* (2022) also reported the similar work on maize against *Fusarium verticilliodes* and identifies eight genotypes AH1625, BAU-MH-18-2, GGMH-114, GK 3207, CMH-12-686, CAH 1511, ADH 1619 and FQH-148 with stable resistance.

CONCLUSIONS

Throughout the seasons, maize inbredsBML-100, BML-101, BML-102, BML-103, BML-106, and BML-108 has sown highly resistant reaction with disease score of less than 3.0 on 1.0 to 9.0 scale. These inbreds are highly useful for utilization in breeding programmes to resistant reaction against charcoal rot of maize with better yields and tropical and subtropical parts of India with semiarid climatic nature and monsoon with intermittent dry spells.

Conflict of Interest. None.

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