Original Research Article

Identification of Maize Genotypes with Charcoal Rot Resistance Suitable for Northern region of Telangana

Comment [H1]: Screening of Some Maize Cultivars for Charcoal Rotin Northern region of Telangana

ABSTRACT

Maize, a vital cereal globally, ranks alongside rice and wheat. Among the significant factors curbing maize yields, post-flowering stalk rot (PFSR) stands as a highly destructive ailment. PFSR leads to yield reductions in maize from 10% to 42%, occasionally even 100% in specific regions. This emphasizes the potential for elevating India's corn production by developing and cultivating PFSR-resistant hybrids. In this study, 37 maize genotypes were evaluated for PFSR resistance, induced by *Macrophominaphaseolina*, using the toothpick inoculation method at Agricultural College, Polasa, Jagtial. These genotypes consisting of 24 single-cross hybrids, 10 parents (6 lines × 4 testers) and three checks. Screening outcomes showcased a spectrum of disease reactions, ranging from high resistance (score 1) to moderate susceptibility (score 7) against PFSR caused by *M. phaseolina*. Out of 37 maize genotypes, one inbred line (PFSR 135), three testers (CML 286, CML 451, and BML 7) and eight hybrids (PFSR 51 × BML 6, PFSR 132 × CML 286, PFSR 29 × CML 451, PFSR 70 × BML 6, PFSR 76 × CML 286, PFSR 135 × CML 286, PFSR 70 × BML 7, PFSR 76 × BML 7) exhibited resistant reaction to the disease. These parent lines and hybrids can be exploited further for development of potential maize hybrids with charcoal rot resistance.

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Keywords: Maize, post flowering stalk rot, *Macrophominaphaseolina*, charcoal rot.

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Introduction

Maize holds paramount importance globally as a cereal crop cultivated across diverse agro-ecologies, contributing significantly to food security, especially in developing nations. In India, maize ranks as the third major cereal crop after rice and wheat. Worldwide, maize occupies 193.7 mha, yielding 1147.6 mtons with a productivity of 5920 kg ha^{-1}. In India, it spans 9.89 mha, yielding 31.65 mtons with a productivity of 3199 kg ha^{-1} (Indiastat, 2021) (1). Despite substantial production, Indian corn yields fall short compared to major global producers. In Telangana, maize thrives across 2.59 lakh ha, yielding 1.76 mtons with a productivity of 6782 kg ha^{-1}, double the national average (Indiastat, 2021) (1). This exemplifies the potential for elevating India's corn production through cultivation of resilient

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hybrids to both biotic and abiotic stresses, understanding yield-contributing genetic traits and adoption of enhanced agronomic practices.

In India, among the biotic stresses affecting maize crop, recent studies (Ramesha*et al.*, 2017) (2) have reported eight fungi and three bacteria responsible for causing stalk rots. The post-flowering stalk rot complex, a group of diseases significantly impactful and widespread, emerges due to distinct soil-borne fungal pathogens: Fusarium stalk rot (*Fusariumverticillioides*), Charcoal rot (*Macrophominaphaseolina*), Black Bundle diseases (*Cephalosporiumacrimonium*) and Late wilt (*Cephalosporium maydis*), as reported by Ramesha*et al.*,(2017) (2).

Manifestations of stalk rot become apparent during the post-flowering and pre-harvest phases of maize development (Ramesha*et al*. 2017) (2). Of these post-flowering stalk rots, charcoal rot, instigated by *M. phaseolina*, stands out as a challenging plant pathogen. Its invasive ability extends to over 500 crucial agricultural crops, including common bean, soybean, chickpea, sunflower, geranium, tomato and sorghum, according to Kaur *et al*. (2012) (3) and Jordaan*et al*. (2019) (4).

Within maize, charcoal rot infection triggers a spectrum of symptoms encompassing seedling blight, stem rot, collar rot and root rot. These affects stem from the visible charcoal-like appearance exhibited by infected plant parts (Kaur *et al*. 2012)(3). The vulnerability of maize plants to *M. phaseolina* infection is increased by elevated temperatures and diminished soil moisture levels. Under favorable conditions, yield losses caused by this pathogen can range from 10 to 42% (Desai et al., 1991) (5), 25.0 to 32.2% (Krishna et al., 2013) (6)and in recent years yield reduction has been reported to be as high as 22.3 o 63.5% (AICRP, 2014) (7) and occasionally even escalating to complete crop failure and retains its pathogenic potency for as long as three years in the soil subsequent to host plant decomposition(Kaur *et al*. 2012)(3).

Globally, researchers are striving to manage charcoal rot, which significantly reduces post-rainy maize yields. A major challenge is the lack of disease-resistant post-rainy maize varieties/ hybrids. To address this issue, it is crucial to identify and develop maize hybrids that exhibit resistance to the pathogen causing charcoal rot. Therefore, the primary approach of the present study toidentify PFSR resistant maize inbred parents and hybrids.

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MATERIALS AND METHODS

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The maize plants showing typical charcoal rot symptoms were collected from Maize Research Center (MRC), Rajendranagar during *Kharif*2022. For isolation of the pathogen diseasedregion of stalkand root portions were washed properly under running tap water to remove excess soil adhered to the root zone and dried on blotter paper before isolation to avoid contamination. The infected parts were then cut into small pieces along with healthy portionwith the help of sterilized blade.These pieces were surface sterilized by dipping for one minute in 1% sodium hypochlorite solution and followed by sterilization to remove traces of sodium hypochlorite. Then these cut bits were dried on blotter paper. A single bit was aseptically transferred to petri plates containing sterilized PDA and incubated for 3-4 days at 28±2°C. Pure cultures were developed through the hyphal tips of fungal mycelia grown out from the infected bits by aseptically transferred to sterile PDA medium. The pathogen *M. phaseolina*was identified based on the descriptions given by Gopala *et al*.(2016) (8).

Inoculum Preparation:

Among various methods of field inoculation, toothpick inoculation is the standard method which was followed in the present study. Round bamboo toothpicks of about 6.5 cm long were boiled three times (about 1 h each time) in tap water to remove gum and resin like toxic substances which might inhibit the growth of the test fungi. After each boiling these were thoroughly washed in fresh water and dried in the sun. After drying, by keeping the tapering end upward, toothpicks were loosely packed in bundles and put into screw capped glass jars/bottles. Prior to autoclaving, potato dextrose broth containing Peptone (1%) and Honey (5%) was added (Banoth*et al*., 2021) (9). The level of broth was adjusted to one-third length of the toothpicks and autoclaved. Subsequently, the sterilized toothpicks were seeded with fungus and incubated 28±2°C for 13 days. Abundant mycelial growth was spread on the toothpicks and inoculum became ready for use in about 13 days.

Field Inoculation:

A total of 37 maize genotypes were used in the field screening studies during *Rabi* 2022. Six lines and four testers were collected from the MRC in Hyderabad during *Kharif* 2022, 24 Single Cross Hybrids (SCHs) developed from these genotypes (6 lines and 4 testers) using a specific mating design known as Line × Tester method, were evaluated along with three checks (Yield, susceptible and resistant checks). These 37 genotypes were sown in two replications, each replication containstwo rows of 4.0 m length. Maize seeds were planted in each replication by following 45 × 20 cm spacing.

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Comment [H19]: These picks were thoroughly washed in fresh water and dried under the sun. After drying, by keeping the tapering end upward, toothpicks were loosely packed in bundles and put into screw capped glass bottles. Prior to autoclaving, potato dextrose broth containing Peptone (1%) and Honey (5%) was added [9]. The level of broth was adjusted to one-third length of the toothpicks and autoclaved. Subsequently, the sterilized toothpicks were seeded with fungus and incubated at 28±2°C for 13 days. Abundant mycelial growth was spread on the toothpicks and inoculum became ready for use in about 13 days.

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Inoculation of themaize genotypes was done at 45-50 days after sowing just before flowering by toothpick method(Anon 1983 and 2012) (10). Before inoculation, a jabber was made by driving/fixing a nail of toothpick size into a wooden handle. For inoculation of the plants, the lower internode (second or third) above soil level was selected in the plants. Then the pointed head of the nail was pushed carefully into the selected internode to make a hole of desired length (2cm). The round toothpick bearing inoculum was inserted into the hole that effectively sealed the hole to prevent drying of the inoculum.

Typical symptoms like partial or whole plant drying appear in the inoculated plants about 20-25 days post-inoculation (DPI). The disease severity of PFSR was recorded at harvesting stage following 1-9 rating scale of Payak and Sharma (1983) (11). Maize stalks collected from field were cleaned and split longitudinally into two equal halves and the score was given based on the disease reaction shown by the maize genotypes.

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Table 1. List of maize inbred lines and crosses used for identification of PFSR resistant and susceptible genotypes

S.No	Maize genotypes	S.No	Maize genotypes
	Lines	19	PFSR 70 × CML 451
1	PFSR 29 (F)	20	PFSR 76 × CML 451
2	PFSR 51 (F)	21	PFSR 132 × CML451
3	PFSR 70 (F)	22	PFSR 135 × CML 451
4	PFSR 76 (F)	23	PFSR 29 × BML 6
5	PFSR 132 (F)	24	PFSR 51 × BML 6
6	PFSR 135 (F)	25	PFSR 70 × BML 6
	Testers	26	PFSR 76 × BML 6
7	CML 286 (M)	27	PFSR 132 × BML 6
8	CML 451 (M)	28	PFSR 135 × BML 6
9	BML 6 (M)	29	PFSR 29 × BML 7
10	BML 7 (M)	30	PFSR 51 × BML 7
	Crosses	31	PFSR 70 × BML 7
11	PFSR 29 × CML 286	32	PFSR 76 × BML 7
12	PFSR 51 × CML286	33	PFSR 132 × BML 7
13	PFSR 70 × CML 286	34	PFSR 135 × BML 7
14	PFSR 76 × CML 286		**Checks**
15	PFSR 132 × CML286	35	Resistant check (PFSR-3)
16	PFSR 135 × CML 286	36	Susceptible check (KM-2)
17	PFSR 29 × CML451	37	Yield check (DHM 117)
18	PFSR 51 × CML 451		

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Table 2. Disease rating scale for scoring disease severity of PFSR in maize genotypes (Payak and Sharma 1983) (11).

Disease rating scale	Disease severity percentage(%)	Disease reaction
1	Healthy or trace/slight discoloration at the site of inoculation	Immune reaction
2	Upto 50% of the inoculated internode is discoloured	Resistant(score:≤3.0)
3	51-75% of inoculated internode is discoloured	
4	76-100% of the inoculated resistant internode is discoloured	Moderately resistant(Score: 3.1-5.0)
5	Less than 50% discolouration of the adjacent internode	
6	More than 50% discolouration of the adjacent internode	Moderately susceptible (Score: 5.1-7)
7	Discolouration of three internodes	
8	Discolouration of four internodes	Susceptible (Score: ≥7.0)
9	Discoloration of five or more internodes and premature death of plant.	

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Table 3. Disease incidence of *Macrophomina* stalk rot recorded in maize genotypes during Rabi 2022-23

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Parent/Cross	In field (Toothpick method)		Grain yield (g/plant)
	Score	Disease reaction	
PFSR 29 × CML 286	7	MS	126.17
PFSR 51 × CML286	5	MR	127.33
PFSR 70 × CML 286	5	MR	145.43
PFSR 76 × CML 286	3	R	106.90
PFSR 132 × CML286	2	R	138.20
PFSR 135 × CML 286	3	R	106.20
PFSR 29 × CML451	2	R	134.47
PFSR 51 × CML 451	7	MS	113.87
PFSR 70 × CML 451	6	MS	120.67
PFSR 76 × CML 451	6	MS	122.03
PFSR 132 × CML451	6	MS	135.20
PFSR 135 × CML 451	6	MS	145.30
PFSR 29 × BML 6	7	MS	112.57
PFSR 51 × BML 6	1	R	125.97
PFSR 70 × BML 6	2	R	115.33
PFSR 76 × BML 6	6	MS	126.33
PFSR 132 × BML 6	5	MR	160.33
PFSR 135 × BML 6	5	MR	152.67
PFSR 29 × BML 7	4	MR	141.40
PFSR 51 × BML 7	6	MS	136.30
PFSR 70 × BML 7	3	R	130.33
PFSR 76 × BML 7	3	R	131.33
PFSR 132 × BML 7	5	MR	128.17

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PFSR 135 × BML 7	4	MR	131.83
PFSR 29 (F)	7	MS	93.10
PFSR 51 (F)	6	MS	63.90
PFSR 70 (F)	5	MR	50.37
PFSR 76 (F)	7	MS	110.60
PFSR 132 (F)	5	MR	81.17
PFSR 135 (F)	1	R	87.17
CML 286 (M)	2	R	102.17
CML 451 (M)	3	R	103.90
BML 6 (M)	5	MR	101.60
BML 7 (M)	3	R	95.60
Resistant check (PFSR 3)	6	MS	93.53
Susceptible check (KM-2)	7	MS	123.60
Yield check (DHM 117)	4	MR	125.80
CV	15.02		4.38
CD (5%)	0.94		8.38

Table 4.Disease score, disease rection, grain yield of top five maize hybrids evaluated during Rabi 2022-23

Hybrids/checks	Disease Score	Disease rection	Grain yield (g/plant)	Yield superiority over yield check	Yield superiority over resistant check	Yield superiority over susceptible check
PFSR 51 × BML 6	1	R	125.97	0.17	32.44	2.37
PFSR 29 × CML451	2	R	134.47	8.67	40.94	10.87
PFSR 132 × CML286	2	R	138.20	12.4	44.67	14.6
PFSR 70 × BML 7	3	R	130.33	4.53	36.8	6.73
PFSR 76 × BML 7	3	R	131.33	5.53	37.8	7.63
Susceptible check (KM-2)	7	MS	123.60	-	-	-
Resistant check (PFSR 3)	6	MS	93.53	-	-	-
Yield check (DHM 117)	4	MR	125.80	-	-	-

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Results and Discussion:

The present work was carried out to identify charcoal rot resistant maize genotypes suitable to cultivate in *Rabi* or Post rainy season in Northern Telangana Zone.A total of 37 maize genotypes includes 6 lines, 4 testers and their 24 F_1 hybrids with three checks were evaluated under artificial inoculation conditions through toothpick method. The charcoal rot disease severity of the tested maize genotypes was assessed in the field using a scale ranging from 1 to 9, as given by Payak and Sharma in 1983 (7).The genotypes displayed various levels of disease reactions, ranging from resistance (score 1) to moderate susceptibility (score 7) against *M. phaseolina*. The data on disease severity collected from the field was summarized and presented in Table 3. Whenever differences observed in score in between the plants of the test entry, the higher value was considered for scoring.

The fieldobservations of screening study revealed that the susceptible check Karimnagar Makka 2 showed typical charcoal rot symptoms measuring score 7 on 1-9disease rating scale with discoulration of three internodes of stalk. In indicates sufficient disease pressure is present in the maize field during the evaluationseason.

Among the evaluated parental inbredsand hybrid, the inbred linePFSR 135 and the hybrid PFSR 51 × BML 6 were found to be highly resistant to charcoal rot with slight discoulration at the site of inoculation.

Out of four testers, one tester BML 6 found moderately resistant and remaining three testers exhibited resistance reaction against charcoal rot disease with disease score ranging from 2 (CML 286) to 3 (BML 7 and CML 451) on 1-9 disease rating scale. Hybrids developed from BML 6 revealed variable disease reaction ranging from 1 (Immune reaction) to 7 (Moderately susceptible) on 1-9 disease rating scale, when crossed with lines PFSR 51(score 1 on 1-9 scale), PFSR 70 (score 2 on 1-9 scale), PFSR 132 and PFSR 135 (score 5 on 1-9 scale), PFSR 76 (score 6 on 1-9 scale) and PFSR 29 (score 7 on 1-9 scale)in comparison to othertwotesters used in present investigation.

Among the 6 hybrids produced in the study from the testers CML 286 and BML 7, the highest disease scores were exhibited when crossed with the individual lines PFSR 29 (scoring 7 on a scale of 1-9) and PFSR 51 (scoring 6 on a scale of 1-9) – both of which displayed a moderately susceptible reaction. Nevertheless, disease resistance reactions were identified in five hybrids originating from both CML 286 and BML 7, marked by evident internode discoloration of up to 75%. Among these five hybrids, the lowest disease severity (scoring 2 on a 1-9 scale) was documented in the PFSR 132 × CML 286 hybrid. These findings also highlight that, hybrids produced through the crossing of PFSR 76 with the two

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parent testers (CML 286 and BML 7) displayed resistance, manifesting a disease score of 3 on the 1-9 scale. Similarly, the hybrids PFSR 135 × CML 286 and PFSR 70 × BML 7 both achieved a disease score of 3 on the 1-9 scale, further affirming their resistance. The remaining fivehybrids developed from the testers CML 286 and BML 7 showed moderate disease resistance reaction withscore 4(PFSR 29 × BML 7 and PFSR 135 × BML) to score 5(PFSR 51 × CML 286; PFSR 70 × CML 286 and PFSR 132 × BML 7) on 1-9 disease rating scale.

Furthermore, all hybrids developed fromtester CML 451 showed moderately susceptible reaction withmore than 50% discoloration of the adjacent internode from the point of inoculation except PFSR 29, which exhibited resistance disease reaction (score 2 on 1-9 disease scale) with less than 50% discouraltion of inoculated internode. While crossing with PFSR 51 showed maximum score 7(1-9 scale) and with PFSR 70, PFSR 76, PFSR 132 and PFSR 135 showed score 6 (1-9 scale).

Among thelines, PFSR 76, PFSR 29 with score of 7 and PFSR 51 with score of 6exhibited moderatesusceptible reaction. Whereas, PFSR 70 and PFSR 132 with score 5 is found to be moderately resistant. The line PFSR 135 (score 1) is found to be highly resistant with slight discoloration at the site of inoculation.

In summary, after conducting a comprehensive screening process involving 37 diverse maize genotypes against the *M. phaseolina*pathogen, the inbred line PFSR 135, displayed significant resistance to post-flowering stalk rot. In addition, three testers*viz.*,CML 286, CML 4541, and BML 7were also exhibited resistance reaction to the disease.

Moreover, the screening extended to investigating hybrid combinations, and it was revealed that eight hybrids demonstrated resistance to post-flowering stalk rot. These hybrids include PFSR 51 × BML 6, PFSR 132 × CML 286, PFSR 29 × CML 451, PFSR 70 × BML 6, PFSR 76 × CML 286, PFSR 135 × CML 286, PFSR 70 × BML 7, and PFSR 76 × BML 7. Among these eight hybrids, five hybrids with resistant disease reaction and score ranging from 1-3 (1-9 scale) have higher grain yield than the susceptible check (KM-2)(Table 4). These findings hold significant promise for enhancing maize crop resilience against *M. phaseolina* and they open up opportunities for further research and breeding efforts to combat this pathogen in maize cultivation.

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References

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