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IDENTIFICATION OF ELITE MAIZE (*Zea mays* L.) GERMPLASM WITH RESISTANCE TO POST-FLOWERING STALK ROT THROUGH PHENOTYPIC FIELD EVALUATION

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ABSTRACT

Post-Flowering Stalk Rot (PFSR) is one of the most destructive diseases limiting maize (*Zea mays* L.) productivity in tropical and subtropical regions, causing severe stalk deterioration, lodging and substantial yield losses. The development and deployment of resistant germplasm remain the most sustainable and economically viable strategy for disease management. The present study was undertaken to identify elite maize germplasm with resistance to PFSR through phenotypic field evaluation under artificial epiphytotic conditions. A diverse set of maize germplasm comprising inbred lines, hybrids and standard checks was evaluated in an alpha lattice design with two replications in two seasons. Artificial inoculation was performed at the post-silking stage using the toothpick inoculation method with a virulent isolate of *Macrophomina phaseolina* and disease severity was assessed using the standard 1–9 disease rating scale. Significant variation in disease response was observed among the evaluated germplasm, indicating the presence of substantial genetic variability for PFSR resistance. Among 126 germplasm evaluated, 22 were classified as resistant, four as susceptible and one is highly susceptible based on pooled disease score. The resistant germplasm exhibited minimal stalk discoloration and lower disease severity, whereas susceptible entries showed extensive stalk degradation and lodging. The resistant germplasm identified in the present investigation represents valuable genetic resources for Maize improvement programmes and can be effectively utilized as donor parents in breeding for durable PFSR resistance. Phenotypic field screening under artificial inoculation proved to be an efficient and reliable approach for identifying resistant sources, thereby facilitating the development of high-yielding maize hybrids with enhanced resistance to post-flowering stalk rot.

Keywords **:** *Zea mays* L., Post-Flowering Stalk Rot, *Macrophomina phaseolina*, phenotypic screening, germplasm evaluation, disease resistance, Maize

Introduction

Maize (*Zea mays* L.) is one of the most important cereal crops globally, serving as a staple food, livestock feed and raw material for numerous industrial products. Owing to its high yield potential, wide adaptability and diverse end uses, it plays a crucial role in ensuring food and nutritional security (Shiferaw *et al*.,2011; Erenstein *et al*.,2022). India is one of the major producing countries, where the crop is cultivated across diverse agro-climatic regions. However, its productivity is frequently constrained by several biotic and abiotic stresses, among which Post-Flowering

Stalk Rot (PFSR) is considered one of the most devastating diseases affecting grain yield and crop standability.

Globally, Maize is the leading cereal crop in terms of production and is cultivated in more than 160 countries. Its versatility and high productivity have made it an indispensable component of global food and nutritional security FAOSTAT (2004). In India, maize is the third most important cereal crop after rice and wheat, occupying about 10.7 million hectares with an annual production of over 43 million tons. The crop plays a vital role in food, feed, fodder and industrial sectors and serves as a valuable raw material for starch, edible oil, ethanol, biofuel, pharmaceuticals, paper, textiles and several other value-added products.

Diversity and exhibits wide variation in plant architecture, maturity duration, grain characteristics and tolerance to biotic and abiotic stresses (Hallauer *et al*., 2010). This rich genetic variability provides immense opportunities for crop improvement through selection and breeding. The development of superior inbred lines with desirable agronomic traits and disease resistance is fundamental to modern maize breeding programmes, as these lines serve as the basic parental material for hybrid development.

Post-Flowering Stalk Rot is a disease complex caused by several fungal pathogens, including *Macrophomina phaseolina*, *Fusarium verticillioides*, *Fusarium graminearum*, *Stenocarpella maydis* and *Cephalosporium acremonium*. Among these, *Macrophomina phaseolina* is one of the most prevalent and destructive pathogens in tropical and subtropical maize-growing regions. The pathogen is soil-borne, survives in the form of microsclerotia for extended periods, and infects plants primarily under conditions of high temperature, moisture deficit and physiological stress. Such environmental conditions favor disease development, leading to severe stalk deterioration, lodging, poor grain filling and substantial reductions in grain yield. (Lal et al., 1980; Sharma and Payak, 1990).

Materials and Methods

Experimental material

The experimental material comprised 126 Maize (*Zea mays* L.) germplasm along with appropriate checks obtained from the Maize Research Centre (MRC), Agricultural Research Institute (ARI), PJTAU, Rajendranagar, Hyderabad. The germplasm was selected based on its genetic diversity and breeding importance for evaluation against Post-Flowering Stalk Rot (PFSR) caused by *Macrophomina phaseolina* under artificial inoculation.

Table 1: List of 126 germplasms of Maize studied during *Kharif* 2025 and *Rabi* 2025-26

S.NO	CODE	GENOTYPE
1	HG	HG-1
2	HG	HG-2
3	HG	HG-3
4	HG	HG-4
5	HG	HG-5
6	HG	HG-6
7	HG	HG-7
8	HG	HG-8
9	HG	HG-9
10	HG	HG-10
11	HG	HG-11
12	HG	HG-12
13	HG	HG-13
14	HG	HG-14
15	HG	HG-15
16	HG	HG-16
17	HG	HG-17
18	HG	HG-18
19	HG	HG-19
20	HG	HG-20
21	HG	HG-21
22	HG	HG-22
23	HG	HG-23
24	HG	HG-24
25	HG	HG-25
26	HG	HG-26
27	HG	HG-27
28	HG	HG-28
29	HG	HG-29
30	HG	HG-30
31	HG	HG-31
32	HG	HG-32
33	HG	HG-33
34	HG	HG-34
35	HG	HG-35
36	HG	HG-36
37	HG	HG-37
38	HG	HG-38
39	HG	HG-39
40	HG	HG-40
41	HG	HG-41
42	HG	HG-42
43	HG	HG-43
44	HG	HG-44
45	HG	HG-45
46	HG	HG-46
47	HG	HG-47
48	HG	HG-48
49	HG	HG-49
50	HG	HG-50
51	HG	HG-51
52	HG	HG-52
53	HG	HG-53

S.NO	CODE	GENOTYPE
54	HG	HG-54
55	HG	HG-55
56	HG	HG-56
57	HG	HG-57
58	HG	HG-58
59	HG	HG-59
60	HG	HG-60
61	HG	HG-61
62	HG	HG-62
63	HG	HG-63
64	HG	HG-64
65	HG	HG-65
66	HG	HG-66
67	HG	HG-67
68	HG	HG-68
69	HG	HG-69
70	HG	HG-70
71	HG	HG-71
72	HG	HG-72
73	HG	HG-73
74	HG	HG-74
75	HG	HG-75
76	HG	HG-76
77	HG	HG-77
78	HG	HG-78
79	HG	HG-79
80	HG	HG-80
81	GP	GP-3
82	GP	GP-7
83	GP	GP-9
84	GP	GP-9A
85	GP	GP-12
86	GP	GP-21-1
87	GP	GP-25
88	GP	GP-26
89	GP	GP-28
90	GP	GP-29

S.NO	CODE	GENOTYPE
91	GP	GP-31
92	GP	GP-33
93	GP	GP-34
94	GP	GP-36
95	GP	GP-37
96	GP	GP-38
97	GP	GP-40
98	GP	GP-41
99	GP	GP-47
100	GP	GP-48
101	GP	GP-51
102	GP	GP-51-1
103	GP	GP-52
104	GP	GP-53
105	GP	GP-54
106	GP	GP-58
107	GP	GP-60
108	GP	GP-68
109	GP	GP-69
110	GP	GP-70
111	GP	GP-71
112	GP	GP-73
113	GP	GP-74
114	GP	GP-75
115	GP	GP-76
116	GP	GP-77
117	GP	GP-80
118	GP	GP-82
119	GP	GP-83
120	GP	GP-85
121	CML	CML-286
122	CML	CML-451
123	DHM	DHM-117
124	DHM	DHM-182
125	DHM	DHM-206
126	EKKA	EKKA-2288

Experimental Site

The field experiment was conducted at the Maize Research Unit (MRU), Agricultural Research Institute (ARI), Professor Jayashankar Telangana Agricultural University (PJTSAU), Rajendranagar, Hyderabad, during the *Kharif 2025* and *Rabi* 2025–2026 seasons. The experimental site is characterized by a tropical semi-arid climate, which is favorable for the development of stalk rot under artificial inoculation.

Multiplication of Pathogen Inoculum

The pathogen, *Macrophomina phaseolina*, was isolated from naturally infected maize stalks exhibiting typical symptoms of post-flowering stalk rot. Small pieces of infected stalk tissue were surface sterilized with 0.1% mercuric chloride for one minute, followed by repeated washing with sterile distilled water. The sterilized tissue pieces were transferred aseptically onto Potato Dextrose Agar (PDA) medium in sterilized Petri plates and incubated at 25 ± 2°C for fungal growth.

Pure cultures obtained from the initial isolation were transferred to fresh PDA slants to maintain stock cultures. Sterile wooden toothpicks placed in bottles containing potato dextrose broth were inoculated with actively growing fungal mycelium under aseptic conditions and incubated at 30–35°C for 7–10 days until the toothpicks were completely colonized by the pathogen. These colonized toothpicks served as inoculum for artificial field inoculation.

Artificial Inoculation Technique

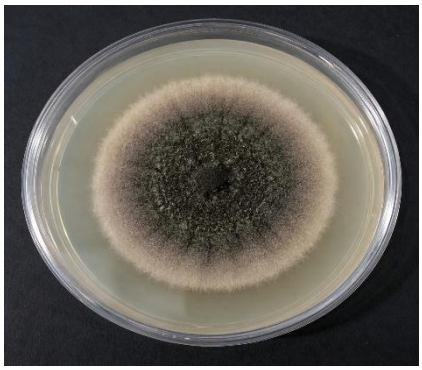

Fig. 1 : The fungal isolate produced a rapidly growing gray to black colony on Potato Dextrose Agar (PDA) with abundant aerial mycelium and black microsclerotia, which are characteristic of *Macrophomina phaseolina.*

Artificial inoculation was carried out using the toothpick inoculation technique at the flowering stage, between tasseling and silking. A small hole (approximately 2 cm deep) was made in the second or third internode above the soil surface using a sterilized jabber fitted with a nail of toothpick diameter. A pathogen-colonized toothpick was inserted into the hole and left in position to facilitate infection. The inoculated plants were monitored regularly, and disease symptoms generally developed 20–25 days after inoculation.

Fig. 2 : Toothpick insertion into internode of maize stem

Experimental Design and Crop Management

The experiment was laid out in an Alpha Lattice Design with two replications. Each genotype was planted in two rows of 2 m length, maintaining a spacing of 60 cm between rows and 20 cm between plants. Recommended agronomic and plant protection practices were followed uniformly throughout the crop growth period.

Disease Assessment

Disease severity was recorded at physiological maturity by splitting the stalk longitudinally and observing the extent of internal stalk discoloration and tissue degradation. Disease reaction of each genotype was assessed using the 1–9 disease rating scale developed for post-flowering stalk rot.

The disease score of individual plants was recorded, and the mean disease score for each genotype was calculated. Based on the average disease score, genotypes were classified into different reaction categories such as Highly Resistant (HR), Resistant (R), Moderately Resistant (MR), Moderately Susceptible (MS), Susceptible (S) and Highly Susceptible (HS) according to the standard PFSR disease rating scale.

Recording of Observations

Observations on disease severity were recorded from all representative plants in each experimental plot. The mean disease score of each genotype was computed and used for phenotypic evaluation. Genotypes exhibiting consistently low disease scores across replications and seasons were identified as potential sources of resistance against post-flowering stalk rot.

Fig. 3 : Resistant genotypes showing minimum pitch disintegration.

Fig. 4 : Susceptible genotypes showing heavy pitch disintegration

Results and Discussion

The disease severity data recorded from each season were subjected to analysis of variance (ANOVA) appropriate for the Alpha Lattice Design to determine the significance of genotypic differences. Adjusted treatment means were estimated by considering block effects within replications. The significance of genotype effects was tested using the appropriate error mean square obtained from the lattice analysis (Patterson and Williams, 1976; Federer, 1998; Gomez and Gomez, 1984).

Table 2 : ANOVA for Disease Score

Source	Df	SS	MS	F	Pr(>F)	Significance
Location	1	0.286	0.286	0.358	0.5504	NS
Treatments	125	230.214	1.842	2.306	0.000368	**
Location: Rep	2	5.147	2.573	3.223	0.04	*
Location: Trt	125	143.464	1.148	1.437	0.0101	*
Loc: Rep: Block	35	65.925	1.884	2.359	0.00009	**
Residual	215	171.679	0.799	-		-

The pooled alpha lattice ANOVA revealed highly significant differences among treatments ($P < 0.01$), indicating the presence of substantial genetic variability for resistance to PFSR among evaluated germplasms (Hooda *et al.* (2017), Kumar *et al.* (2016) and Rashid *et al*. (2021). The effects of location were non-significant, suggesting that the overall performance of the genotypes was consistent across environments. However, significant Location × Treatment interaction indicated differential responses of genotypes across locations. Significant Location × Replication and Location × Replication × Block effects further confirmed the effectiveness of the alpha lattice design in accounting for environmental heterogeneity and improving experimental precision

Summary of statistics

Grand Mean (3.815):

The grand mean represents the overall average disease score of all maize germplasm evaluated across locations and replications.

Coefficient of Variation (CV = 23.43%):

The coefficient of variation indicates the relative variability in the experimental data. A CV of 23.43% suggests moderate experimental variation.

Standard Error of Mean [SEm (±) = 0.632]:

The standard error of the mean measures the precision of the treatment mean estimates. A lower SEm indicates greater precision in comparing genotype means.

Critical Difference [CD (P = 0.05) = 1.76]:

The critical difference represents the minimum difference required between two treatment means for them to be considered significantly different at the 5% probability level.

Error Mean Square (MSE = 0.799):

The error mean square estimates the experimental error remaining after accounting for treatment and design effects and is used in the calculation of SEm and CD.

Error Degrees of Freedom (df = 215):

The error degrees of freedom represent the independent observations used to estimate the experimental error, providing the basis for statistical tests such as the F-test and critical difference.

Table 3 : Elite Maize Germplasm identified resistance to PFSR

S.no	Germplasm	Kharif Score	Rabi Score	Pooled Score	Disease Reaction
1	GP-33	2.75	2	2.375	Resistant
2	DHM-117	2.75	2.25	2.5	Resistant
3	HG-69	2.75	2.5	2.5	Resistant
4	GP-37	2.25	3	2.625	Resistant
5	HG-33	2.75	2.5	2.625	Resistant
6	HG-7	3.25	2.25	2.75	Resistant
6	HG-38	2.75	2.75	2.75	Resistant
7	HG-2	3.25	2.25	2.75	Resistant
8	HG-57	3.25	2.25	2.75	Resistant
9	HG-11	3.25	2.25	2.75	Resistant

10	DHM-182	3.25	2.25	2.75	Resistant
11	HG-1	3.5	2.25	2.875	Resistant
12	HG-12	3	2.75	2.875	Resistant
13	HG-15	3	2.75	2.875	Resistant
14	HG-61	3	2.75	2.875	Resistant
15	HG-64	3	2.75	2.875	Resistant
16	HG-70	3.25	3.5	2.875	Resistant
17	HG-8	3.25	2.5	2.875	Resistant
18	HG-9	3	3	3	Resistant
19	HG-14	2.75	3.25	3	Resistant
20	HG-65	3.25	2.75	3	Resistant
21	HG-6	3	3.25	3.125	Resistant
22	HG-36	3.5	3.25	3.125	Resistant

Table 4 : Susceptible Maize Germplasm identified

S.No	Germplasm	Kharif Score	Rabi Score	Pooled Score	Disease Reaction
1	HG-32	7.75	6.25	7	Highly susceptible
2	HG-16	6	6.5	6.25	Susceptible
3	HG-28	6.5	5.5	6	Susceptible
4	HG-52	6.25	5.75	6	Susceptible
5	HG-77	6.25	5.5	5.8	Susceptible

Conclusion

Post-Flowering Stalk Rot (PFSR), caused by *Macrophomina phaseolina*, remains a major constraint to sustainable maize production, emphasizing the need for the identification and utilization of resistant germplasm. In the present study, 126 diverse maize germplasm were evaluated under artificial epiphytotic conditions using the toothpick inoculation technique in an alpha lattice design across two seasons. The pooled analysis revealed significant genetic variation for PFSR resistance, demonstrating the effectiveness of phenotypic screening in differentiating resistant and susceptible germplasm.

Based on pooled disease scores, 22 germplasm were identified as resistant, four as susceptible and one is highly susceptible reflecting a wide range of genetic variability for resistance within the evaluated material. The resistant germplasm consistently exhibited low disease severity across both seasons, indicating stable resistance under high disease pressure.

The resistant germplasm identified in this investigation represents valuable genetic resources for maize improvement programmes and can be effectively exploited as donor parents for the development of high-yielding, PFSR-resistant hybrids and inbred lines. Furthermore, the study confirms that artificial inoculation coupled with phenotypic field evaluation provides a robust, reliable and reproducible approach for large-scale screening of maize germplasm against PFSR. These findings contribute to strengthening resistance breeding programmes and support the development of climate-resilient Maize cultivars with improved yield stability and durable resistance to post-flowering stalk rot.

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