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Breeding Resistance for Post Flowering Stalk rot (*Macrophomina phaseolina*) in Maize Identification of Resistance against Post Flowering Stalk Rot (*Macrophomina phaseolina*) in Maize

M. Banoth[1], K. Prabhavathi[2*], D. Bhadru[3] and B. Mallaiah[3]

[1]Department of Genetics and Plant Breeding, Professor Jayashankar Telangana State Agricultural University (PJTSAU), College of Agriculture, Rajendranagar, Hyderabad-500 030, Telangana, India.
[2]Seed Research and Technology Centre, PJTSAU, Rajendranagar, Hyderabad-500 030, Telangana, India.
[3]Agricultural Research Institute, Maize Research Centre, PJTSAU, Rajendranagar, Hyderabad-500 030, Telangana, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Maize is one of the most important staple food crops in the World. However, the yields of maize have been affected by various fungal infestations. Post flowering stalk rot is one of the devastating diseases and so, we planned our study to order to identify suitable resistance maize genotypes against post flowering stalk rot (PFSR) complex caused by *Macrophomina phaseolina* through in-*vivo* screening and toothpick method for creating artificial epiphytotics. A total of 20 maize inbreds were screened and crossed in Line × Tester mating design (15 × 5) during *Kharif* 2019, Six resistant inbred lines were identified and generated the 75 F_1s (SCHs) at MRC, ARI, Rajendranagar,

**Corresponding author: E-mail: konaprabhavati@yahoo.co.in;*

Hyderabad. All these 20 parents and 75 F_1s along with three checks were evaluated by raising the crop in disease sick plot accompanied by toothpick inoculation during *Rabi*, 2019-20, in a Randomized Block Design with two replications. The field screening of maize genotypes by the standard toothpick method which needs about 40 days for expression of plant drying symptoms due to PFSR and data are possible to record only at the time of crop harvesting using 1-9 rating scale of PFSR for scoring disease severity in-*vivo* condition by splitting the stem of each plant. As a result, most of the genotypes were exhibited disease reaction varying from resistant (score 2) to moderately resistant (score 5) against *M. phaseolina*. While studying the genetics of PFSR, we found that interaction of lines and testers were proportionally contributed towards resistant, and degree of dominance is preferably non-additive gene action, it shows that the magnitude of dominance was higher than additive effect indicating that PFSR resistance is largely governed by dominance effect *i.e.,* non additive component is not fixable for resistance. It is also found that the resistant genotypes also exhibited highest significant positive heterosis and combining ability effects (GCA and SCA). A considerable yield reduction in grain yield (10.5 to 28.3%) over checks was observed in susceptible lines. Most of the genotypes were found resistant as the reduction in yield is low. Hybrids developed using such lines exhibited high yields which are promoted for extensive testing to know their stability before release as commercial hybrids.

Keywords: Maize genotypes; toothpick inoculation; post flowering stalk rot; resistance; disease severity scale; gene action; heterosis; combining ability; line × tester mating design.

1. INTRODUCTION

Maize (*Zea mays* L. 2n = 2x = 20) is known as Miracle crop and Queen of cereals because of its highest genetic yield potential among the cereals (dacfw, 2016). The global maize production is about 1.09 billion metric tonnes from 153.0 million hectares [1], (CIMMYT, 2020). The USA has highest productivity (10.57 t ha^{-1}), which is double than the global average (4.92 t ha^{-1}). Whereas, the average productivity in India is about 2.68 t ha^{-1} with production of 24.26 million tonnes from 9.3 million hectares, the country lags far behind in productivity against world average. However, in Telangana State maize is grown in almost all the districts in an area of 0.64 million hectares, with a production of about 2.60 million tonnes [2].

In India, the low performance of potenatial genotypes due to various biotic and abiotic stress is one of the major constraints hindering maize production. Apart from pest and diseases, fungal diseases like, post flowering stalk rots (PFSR) poses a major threat to the productivity of maize [3]. PFSR is a complex disease, which commonly appears when there is scarcity of irrigation coupled with high soil temperature at flowering stage of the crop. PFSR is caused by different fungal pathogens but, charcoal rot or Macrophomina stalk rot (MSR) caused by *Macrophomina phaseolina* is more prevalent and destructive in Telangana State as well as in Rajasthan, Bihar, Andhra Pradesh, Uttar Pradesh, Punjab, Madhya Pradesh and West Bengal. Stalk rot is found to be prevalent in the plains only in the kharif crop when summer temperature becomes relatively high (30° to 35°C). The disease incidence, recorded in India time to time, ranged from 10.0 to 42.0% [4], 25.0 to 32.2 % (Krishna et al. 2013) and in recent years yield reduction has been reported to be as high as 22.3 to 63.5% [5].

In order to combat this problem, development of maize cultivars with genetic resistant represent one of the most cost-efficient, safe and eco-friendly solutions for reducing the yield losses caused by PFSR compared to chemical and biological control methods. Information on the nature of inheritance of PFSR resistance is lacking, which is a prerequisite to initiate appropriate breeding program for the development of PRSR resistant varieties, on which very little emphasis had been made so far. To develop disease resistant varieties, screening of available genotypes against the pathogens was done under artificial epiphytotic condition and it yielded a set of stalk rot resistant germplasm in India [6,7]. In India, artificial epiphytotic condition for stalk rot disease is created by inoculating the plants in the field just after flowering mainly by toothpick method of inoculation [8]. Hence, attempts were made to identify the PFSR resistance hybrids which would enable breeders to formulate sound basis for future breeding programmes.

2. MATERIALS AND METHODS

2.1 Seed Materials

The evaluated seeds of 20 maize genotypes were collected from the Maize Research Centre (MRC), Agricultural Research Institute (ARI), PJTSAU, Rajendranagar, Hyderabad, against PFSR complex and grain yield trait (Table 1).

2.2 In-vivo Screening

During kharif 2019, these selected lines were crossed in an L × T mating design and 75 F1s were obtained. The 20 parental lines were also artificially inoculated with charcoal rot disease material and confirmed their reaction to the disease. During rabi 2019-20, these 75 F1s and 20 parents along with three standard checks (DHM-117, BIO-9544 and KAVERI-50) a total of 98 genotypes were subjected to evaluate by raising the crop in disease sick plot accompanied by toothpick inoculation at MRC, ARI, Rajendranagar (Table 1).

2.3 Multiplication of Inoculum

Artificial inoculation was done with tooth picks on which the disease casual organisms were grown in the laboratory. For this purpose, infected maize stems with PFSR were collected, cut into small bits and surface sterilized with 0.1% mercuric chloride for one minute followed by washing with sterile distilled water. Finally, a single bit was aseptically transferred to sterilized 10 cm Petri plates containing 20 ml of sterilized Potato Dextrose Agar medium (PDA). The plates were incubated for three days at 24±20c. The fungal hyphae were then aseptically transferred to culture tubes containing the sterile PDA medium and incubated for 10 days to get the stock culture of the pathogen. Broth medium was poured under aseptic condition into a sterilized, wide mouthed bottle with screw cap, containing toothpicks. Then from stock culture, two loops of mycelia suspension were seeded in bottle containing toothpicks under aseptic conditions. Then bottles were incubated at 350c for 7 days. The toothpicks covered with abundant mycelia of the fungus were then ready to use in about 10 days in field inoculation.

2.4 Inoculation Technique

Before inoculation, one jabber was made by driving/fixing a nail of toothpick size into a wooden handle. For inoculation, most appropriate plant stage for inoculation is between tasseling and pollination for that the lower internode (second or third) above soil level was selected. 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 inoculums were inserted into the hole that effectively sealed the hole to prevent drying of the inoculums. Typical symptoms like partial or whole plant drying appear in the inoculated plants about 20-25 days post-inoculation (DPI).

2.5 Experimental Designs

Seed of the test lines were sown in two rows plot of 2m length with row to row spacing of 60 cm and plant to plant spacing of 20 cm in a Randomized Block Design. In order to increase the disease pressure in the field, the susceptible local checks were planted on every 10th row and on both sides of plot. Data on disease incidence was collected from each replication in the two rows plot technique in the field during rabi 2019-20.

2.6 Disease Severity Rating

Classification for the reactions for the pathogens was done on an individual plant basis, splitting the stalk open and observing the rot is the most reliable method of determining the amount and extent of stalk rot and the 1-9 index scale, suggested by Sreenu et al. [9] was followed for scoring and scale has been unequally distributed into four categories of disease severity viz., resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible reaction (S) (Table 2).

2.7 Statistical Analysis

Data from field experiments were analyzed using analysis of variance (ANOVA) to determine the contribution of replication, genotypes and their interaction for Randomized Block Design (RBD) by using formula of Panse and Sukhatme [10] and combining ability by using formula of Kempthorne [11].

Table 1. Details of maize inbred lines used in Line ×Tester mating design for identification of PFSR resistant and susceptible genotypes

Genotypes	Pedigree	Source	Grain type	Colour silking
MGC-6	(CML451-B*7/([CML451/CL-RCY016)-B-18-1-1-1-BBB)-B-11-BB	MRC, ARI, Rajendranagar	Flint	Yellow
MGC-9	(((CML161×CML451)-B18-1-BBB/CML161-B)-B13-BB(NonQ)-BBB/CML395/MBRC5BcF114-1-2-3-B-4-2-B)DH-3007-B*4)-B-8-BB	MRC, ARI, Rajendranagar	Flint	Yellow
MGC-15	(AMDROUTI (DT-Tester)C1f2-36-b*5/(POP502C5#18/GEMN-0145)-B-21-2-1-1-B)-B-3-BB	MRC, ARI, Rajendranagar	Flint	Orange
MGC-32	AMDROUT(5×6)C3F2-B2-15-1-BB	MRC, ARI, Rajendranagar	Flint	Yellow
MGC-92	PT9633301-1-B*4-1-B*6-1-BBB-#-BB	MRC, ARI, Rajendranagar	Flint	Yellow
MGC-137	(MARSSYN-155)-4-1-1-BB	MRC, ARI, Rajendranagar	Semi-dent	Orange
MGC-230	CML452=Ac8328BNC6-166-1-1-1-B*15-#-BB	MRC, ARI, Rajendranagar	Flint	Yellow with cap
MGC-237	(POP501C5#8/GEMS-0039)-B-10-1-1--1-BBB	MRC, ARI, Rajendranagar	Flint	Yellow
MGC-238	(CML451/CA00360/P3011F2-3-5-6-1-B*10)-B-4	MRC, ARI, Rajendranagar	Flint	Orange
MGC-239	(CML161×CLQ-RCYQ49=(CML176/CL-G2501)-B-55-2-1-B)-B-19-1-B*11	MRC, ARI, Rajendranagar	Flint	Orange
MGC-242	CML227-B*12	MRC, ARI, Rajendranagar	Semi-flint	Yellow
MGC-248	DTPYC9-F46-3-6-1-2-2-1-2-B*7-B-B	MRC, ARI, Rajendranagar	Flint	Pinkish orange
MGC-252	NEI9008-B*9	MRC, ARI, Rajendranagar	Semi-flint	Yellow with cap
MGC-254	CLQ-RCYQ36-B-1-B*8-B-B	MRC, ARI, Rajendranagar	Semi-dent	Yellow
MGC-256	CA00360/P3011F2-3-5-6-1-B*12	MRC, ARI, Rajendranagar	Flint	Yellow with cap
BML-6	BML-6(SRRL65-b96-1-1-2-#-2-1-1-1-1	MRC, ARI, Rajendranagar	Semi-flint	Yellow
BML-7	BML-7(X2 y pool × CML226-B98R-1-1-1-xb-xb-xb	MRC, ARI, Rajendranagar	Flint	Orange
BML-14	BML-14(COIB96 K-1-#-1-2-xb-xb-1-2-xb-xb-2-xb-xb-xb	MRC, ARI, Rajendranagar	Semi-dent	Pinkish orange
GP-170	Selected from CIMMYT lines	CIMMYT, Mexico	Dent	Yellow with cap
GP-311	Selected from CIMMYT lines	CIMMYT, Mexico	Dent	Yellow with cap
DHM-117	BML-6 × BML-7	MRC, ARI, Rajendranagar	Flint	Yellow with cap
BIO-9544	Bioseed Pvt Ltd.	Bioseed Pvt Ltd.	Flint	Yellow
KAVERI-50	Kaveri Seed Company Ltd.	Kaveri Seed Company Ltd.	Flint	Yellow

MRC: Maize Research Centre, ARI: Agricultural Research Institute

Table 2. Disease rating scale for scoring disease severity of PFSR [9]

Disease rating scale	Disease severity percentage (%)	Disease reaction
1	Healthy or trace/slight discolouration at the site of inoculation	Immune reaction
2	Up to 50% of the inoculated internode is discoloured	Resistant (Score: ≤3.0)
3	51-75% of the 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: 5.1-7.0)
7	Discolouration of three internodes	
8	Discolouration of four internodes	Susceptible (Score: ≥ 7.0)
9	Discolouration of five or more internodes and premature death of plant	

Estimation of general and specific combining ability effects ($X_{ijk} = \mu + g_i + g_j + S_{ij} + r_k + e_{ijk}$)

Estimation of GCA effects (a) for lines ($g_i = X_i$... /tr-X…/1tr) (b) for testers ($g_i = X_j$.. /lr - X…/1tr)

Estimation of SCA effects ($S_{ij} = [X_{ij} / r] – [X_i$.. /tr] – [$X_j$../lr] + [X…/ltr])

Standard heterosis was calculated by the formula (Mean of F1 - Mean of check/Mean of check ×100). Based on these all statistics and rating scale (score 1-9) resistance maize genotypes were identified in present investigation.

3. RESULTS AND DISCUSSION

3.1 Screening of Maize Genotypes in Field During Kharif-2019

Screening was done in selected inbreds by toothpick inoculation, out of the 15 lines screened against *M. phaseolina*, only four lines, *viz*., MGC-237, MGC-248, MGC-254 and MGC-256 were found resistant, four lines, *viz*., MGC-9, MGC-137, MGC-242 and MGC-252 were moderately resistant, five lines, *viz*., MGC-6, MGC-32, MGC-92, MGC-238 and MGC-239 were moderately susceptible and only two lines, *viz*., MGC-15 and MGC-230 were found susceptible. Out of the 5 testers screened against *M. phaseolina*, only two testers, *viz*., BML-6 and GP-311 were found resistant, one tester, GP-170 was moderately resistant, one tester, BML-14 was moderately susceptible and one tester, BML-7 showed susceptibility (Table 3). Simultaneously, these 20 inbred lines were crossed in L × T mating fashion (15 L × 5 T) to generate the 75 F1s and all these 20 parents and 75 F1s along with three standard checks were subjected to evaluate by raising the crop in disease sick plot accompanied by toothpick inoculation during Rabi, 2019-20, in a Randomized Block Design with two replications and found that most of the selected inbreds were shows resistant to moderate resistant with low in limiting grain yield.

3.2 Evaluation and Screening of Maize Genotypes in Field during Rabi-2019-20

The analysis of variance of parents and hybrids for grain yield and PFSR disease score are presented in Table 4. The mean sums of squares due to genotypes (parents and hybrids) were highly significant for both the traits studied in this investigation. Further, interaction between the parents vs. crosses and when the effects of crosses was partitioned into lines, testers and lines x testers effects was found significant for both characters under study (Table 5). Hence it can be concluded that significant variability is present in the material taken up for the study.

The overall means per se of lines, testers and cross combinations revealed that hybrids were registered superior performance than parents with respect to grain yield (kg.ha^{-1}) and disease score (1-9). Further the hybrids in general were tall and high yielding over parents. The hybrids, *viz*., MGC-252 × GP-311, MGC-254 × GP-311, MGC-256 × BML-GP-311, MGC-256 × GP-170, MGC-242 × BML-14, MGC-248 × BML-14 and MGC-254 × GP-170 were recorded higher resistance PFSR with grain yield compared to best check BIO-9544 (Table 3 and Table 8).

The estimates of GCA and SCA variances and their ratios are presented in the Table 6 and observed that SCA variances were higher than GCA variances for both traits studied which indicates predominance of non-additive gene action. It is evident that the predominance of non-additive gene action over the additive gene action is ideal for exploitation through heterosis breeding for getting higher resistance. A comparison of the magnitude of variance components due to gca and sca confirmed the gene action in controlling the expression of traits. The ratio of GCA and SCA variance was less than unity indicating the predominant role of non-additive gene action for both traits under study and the magnitude of dominance was higher than additive effect indicating that PFSR resistance is largely governed by dominance effect i.e. non-additive component is not fixable.

Proportional contribution of lines, testers and line × tester to total variances on performance of hybrids for PFSR resistance with grain yield revealed that line × tester interaction seems to be high in PFSR resistance development and whereas the lines were contributed more in grain yield production (Table 7).

Table 3. Disease incidence and general combining ability of parents recorded in field for traits MSR and grain yield

Parents	Grain yield mean (kg/ha)	General combining ability effect		In field (Toothpick method)	
		Disease score (1-9)	Grain yield (kg/ha)	MSR mean score (1-9)	Disease reaction
Lines					
MGC-6	7504.13	-0.11	-4984.2**	7	MS
MGC-9	7208.30	-0.31	-5036.7**	5	MR
MGC-15	7829.13	-0.31	1227.53**	8	S
MGC-32	7429.13	-1.11**	3889.2**	7	MS
MGC-92	8299.96	-0.31	-1495.77**	6	MS
MGC-137	7649.96	0.28	-1229.1**	4	MR
MGC-230	7258.30	0.28	1588.27**	8	S
MGC-237	7570.80	-0.71**	1584.93**	3	R
MGC-238	8216.63	0.68**	-1534.93**	6	MS
MGC-239	7312.47	0.88**	-1469.1**	7	MS
MGC-242	7962.46	0.68**	1597.43**	5	MR
MGC-248	7533.30	1.08**	1626.6**	3	R
MGC-252	9441.62	-1.11**	1860.77**	4	MR
MGC-254	9062.46	-0.01	1745.77**	3	R
MGC-256	9054.13	0.08	2204.93**	2	R
Testers					
BML-6	8570.79	0.25	-136.44	3	R
BML-7	8516.63	0.95**	-170.88**	8	S
BML-14	8358.29	-0.24	223.27	7	MS
GP-170	8804.13	-1.31**	511.33**	5	MR
GP-311	9496.79	0.35**	572.72**	3	R

** Significant at 5 per cent level; ** Significant at 1 per cent level*

Table 4. Analysis of variance from RBD for PFSR disease score and grain yield

Character	Source of variation		
	Replications (d.f = 1)	Treatments (d.f = 97)	Error (d.f = 97)
Disease score (1-9)	0.25	10.95**	0.51
Grain yield per plant (kg/ha)	201428.20	13939850.00**	793501.90

** Significant at 5 per cent level; ** Significant at 1 per cent level*

Table 5. Analysis of variance from combining ability for PFSR disease score and grain yield

Source of variation	Disease score (1-9)	Grain yield (kg/ha)
Replications (d.f = 1)	0.96	93332.89
Treatments (d.f = 94)	11.03**	14199716.5**
Parents (d.f = 19)	10.82**	939460.90**
Parents vs. Crosses (d.f = 1)	0.40**	165744475.70**
Crosses (d.f = 74)	11.22**	15556480.00**
Lines (d.f = 14)	4.55**	79588700.00**
Testers (d.f = 4)	21.62**	679419.40
Lines × Testers (d.f = 56)	12.15**	611063.10**
Error (d.f = 74)	0.514	835968.70

** Significant at 5 per cent level; ** Significant at 1 per cent level*

Table 6. Estimates of general and specific combining ability variances, proportionate gene action and degree of dominance

Character	Source of variation			Degree of Dominance $(\sigma^2sca/\sigma^2gca)^{1/2}$	Nature of Gene Action
	σ^2gca	σ^2sca	σ^2gca/σ^2sca		
Disease score (1-9)	1.25	5.81	0.21	2.15	Non-Additive
Grain yield (kg/ha)	3932051.30	4052055.60	0.97	1.03	Non-Additive

Table 7. Proportional contributions of lines, testers and line × tester interaction on the performance of hybrids

Character	Proportional contribution (%)		
	Lines	Testers	Line × Tester
Disease score (1-9)	7.68	10.41	81.91
Grain yield (kg/ha)	96.79	0.24	2.97

The gca effects for disease score among the lines ranged from -1.11 (MGC-252 and MGC-32) to 1.08 (MGC-248). Among the testers, gca effects ranged from -1.31 (GP-170) to 0.95 (BML-7). Three lines were recorded significant negative gca effects *viz*., MGC-252 (-1.11), MGC-32 (-1.11) and MGC-252 (-0.71). One tester recorded significant negative gca effects *viz*., GP-170 (-1.31) were good general combiners for Disease resistant. Whereas, four lines and two testers were recorded significant positive gca effects (Table 3). The sca effects ranged from -3.75 (MGC-239 × GP-311) to 4.24 (MGC-252 × GP-311). Hybrid, *viz*., MGC-239 × GP-311 (-3.75) was recorded the highest negative sca effect followed by MGC-254 × GP-311 (-3.55), MGC-256 × GP-311 (-3.15), MGC-252 × GP-311 (-3.15) and MGC-6 × BML-14 (3.15) were found superior to remaining hybrids for disease resistant. Among 75 hybrids, 26 and 27 hybrids were recorded negative and positive significant sca effects respectively (Table 8).

The gca effects for grain yield (kg/ha) among the testers ranged from -170.88 (BML-7) to 572.72 (GP-311). Among the lines, gca effects ranged from -5036.70 (MGC-9) to 3889.20 (MGC-32). Nine lines recorded positive significant gca effects, of which *viz*., MGC-32 (3889.20) was highest followed by MGC-256 (2204.93), MGC-252 (1860.77), MGC-254 (1745.77), MGC-248 (1626.60), MGC-242 (1597.43), MGC-230 (1588.27), MGC-237 (1584.93) and MGC-15 (1227.53) and two testers GP-311 (572.72) and GP-170 (511.33) were record positive significant gca effect and were good combiners for grain yield. Whereas, six lines and tester BML-7 recorded significant negative gca effects (Table 3). The sca effects for grain yield ranged from -3407.55 (MGC-252 × BML-7) to 4179.77 (MGC-252 × GP-311 and MGC-254 × GP-311). 26 hybrids, recorded significant positive sca effects, of which *viz*., MGC-252 × GP-311 and MGC-254 × GP-311 (4179.77) were the highest followed by MGC-256 × GP-311, MGC-256 × GP-170, MGC-242 × BML-14 and MGC-248 × BML-14 were

recorded 3924.77, 3875.60, 2807.55 and 2789.05 respectively and they were superior specific crosses for grain yield. 29 hybrids recorded significant negative sca effects (Table 8).

Standard heterosis for disease score over best check BIO-9544 varied from -88.89 (MGC-252 × GP-311) to 88.89% (11 hybrids were recorded with the same value). Among 75 hybrids, only 20 hybrids *viz*., MGC-254 × GP-311 (-84.44%), MGC-256 × GP-170 (-82.22%), MGC-256 × GP-311 (-80.00%) and MGC-242 × BML-14 (-78.89%) were recorded significant negative heterosis over best check and these hybrids were superior to remaining ones in case of PFSR resistance (Table 8). Standard heterosis for grain yield over best check BIO-9544 varied from -45.10 (MGC-92 × GP-311) to 42.48 (MGC-252 × GP-311 and MGC-254 × GP-311). Among 75 hybrids 27 hybrids were recorded significant positive heterosis over best check. Hybrids, MGC-252 × GP-170 (40.67%), MGC-256 × GP-311 (38.71%), MGC-256 × GP-170 (37.54%), MGC-254 × BML-14 (37.58%) and MGC-242 × BML-14 (37.14%) were recorded high significant positive heterosis over best check and acts as superior to other hybrids for this trait (Table 8).

The disease severity was recorded in the field by using a scale (1-9 cm) of Payak and Sharma [12]. All these maize inbred lines were screened in field by toothpick method of inoculation. As a result, most of the genotypes were exhibited disease reaction varying from resistant (score 2) to moderately resistant (score 5) against *M. phaseolina*.

Screening of 20 parents again in rabi 2019-20, they performed the same results as they were already screened in khari-2019 for PFSR resistance and screening in their F1s and checks, among the 75 SCHs hybrids, 15 hybrids *viz*., MGC-9 × BML-6, MGC-9 × BML-14, MGC-32 × BML-14, MGC-32 × GP-170, MGC-92 × GP-170, MGC-137 × GP-311, MGC-237 × BML-7, MGC-242 × BML-14, MGC-248 × GP-311, MGC-252 × BML-14, MGC-252 × GP-311, MGC-254 × BML-14, MGC-254 × GP-311, MGC-256 × GP-170 and MGC-256 × GP-311 were found resistant, 16 hybrids, *viz*., MGC-6 × BML-7, MGC-6 × BML-14, MGC-6 × GP-170, MGC-15 × GP-311, MGC-32 × BML-6, MGC-32 × GP-311, MGC-137 × GP-170, MGC-230 × BML-7, MGC-230 × GP-311, MGC-238 × BML-7, MGC-239 × BML-14, MGC-239 × GP-311, MGC-242 × GP-170, MGC-248 × BML-14, MGC-252 × BML-6 and MGC-256 × BML-14 were moderately resistant, 22 hybrids, *viz*., MGC-6 × BML-6, MGC-6 × GP-311, MGC-9 × BML-7, MGC-9 × GP-311, MGC-15 × BML-6, MGC-92 × BML-7, MGC-92 × GP-311, MGC-137 × BML-6, MGC-137 × BML-14, MGC-230 × BML-6, MGC-237 × GP-170, MGC-237 × GP-311, MGC-238 × BML-14, MGC-238 × GP-170, MGC-239 × BML-7, MGC-242 × BML-7, MGC-242 × GP-311, MGC-248 × BML-7, MGC-248 × GP-170, MGC-252 × GP-170, MGC-256 × BML-6 and MGC-256 × BML-7 were moderately susceptible, 22 hybrids, *viz*., MGC-9 × GP-170, MGC-15 × BML-7, MGC-15 × BML-14, MGC-15 × GP-170, MGC-32 × BML-7, MGC-92 × BML-6, MGC-92 × BML-14, MGC-137 × BML-7, MGC-230 × BML-14, MGC-230 × GP-170, MGC-237 × BML-6, MGC-237 × BML-14, MGC-238 × BML-6, MGC-238 × GP-311, MGC-239 × BML-6, MGC-239 × GP-170, MGC-242 × BML-6, MGC-248 × BML-6, MGC-252 × BML-7, MGC-254 × BML-6, MGC-254 × BML-7 and MGC-254 × GP-170 were found susceptible and whereas checks, DHM-117, BIO-9544 and KAVERI-50 were found resistant, moderately resistant and susceptible respectively (Table 8).

Disease severity data obtained from the field was summarized based on their average disease reaction presented in the (Table 9). In contrast, the currently followed inoculation procedure developed by Payak and Sharma [12] requires a longer time of about 40 days for expression of plant drying symptoms due to PFSR and data are possible to record only at the time of crop harvesting.

In conclusion, the disease severity of PFSR along with grain yield is recorded in the field by observing the disease symptoms on the whole/individual plant. Hence, in ordered to identified PFSR resistant lines, screening of 98 maize genotypes in field against *M. phaseolina*, only four lines, *viz*., MGC-237, MGC-248, MGC-254, MGC-256 and two testers, *viz*., BML-6 and GP-311. Whereas, 15 crosses *viz*., MGC-9 × BML-6, MGC-9 × BML-14, MGC-32 × BML-14, MGC-32 × GP-170, MGC-92 × GP-170, MGC-137 × GP-311, MGC-237 × BML-7, MGC-242 × BML-14, MGC-248 × GP-311, MGC-252 × BML-14, MGC-252 × GP-311, MGC-254 × BML-14, MGC-254 × GP-311, MGC-256 × GP-170 and MGC-256 × GP-311 were found resistant.

Table 8. Disease incidence, specific combining ability and standard heterosis of hybrids recorded in field for traits MSR and grain yield

Hybrids	Grain yield mean (kg/ha)	Specific combining ability effect		Heterosis over best check (BIO-9544)		In field (Toothpick method)	
		Disease score (1-9)	Grain yield (kg/ha)	Disease score (1-9)	Grain yield (kg/ha)	MSR mean score (1-9)	Disease reaction
Crosses							
MGC-6 × BML-6	14379.1	-1.65**	-453.55	-22.22	-20.87**	7	MS
MGC-6 × BML-7	14517.6	-1.35**	-2274.94*	-22.22	-12.81**	5	MR
MGC-6 × BML-14	14499.94	-3.15**	351.72	-66.67**	-28.75**	4	MR
MGC-6 × GP-170	14604.1	3.91**	-397	66.67**	11.36**	4	MR
MGC-6 × GP-311	14495.77	2.24**	1134.49**	66.67**	-5.66**	6	MS
MGC-9 × BML-6	14112.44	-1.45**	984.77	-22.22	-9.92**	3	R
MGC-9 × BML-7	13879.11	2.84**	-3072.44*	88.89**	22.48**	7	MS
MGC-9 × BML-14	14058.27	-1.95**	-254.11	-44.44**	25.44**	3	R
MGC-9 × GP-170	13658.27	-1.88**	442.27*	-66.67**	16.21**	8	S
MGC-9 × GP-311	13629.11	2.44**	2400.49*	66.67**	-12.13**	7	MS
MGC-15 × BML-6	13654.11	1.54**	-511.88	44.44**	-4.87**	7	MS
MGC-15 × BML-7	14037.44	4.24**	-906.60*	-44.44**	-5.68**	8	S
MGC-15 × BML-14	14199.94	-0.95	-807.55*	-22.22	-38.19**	8	S
MGC-15 × GP-170	15170.77	-0.88	-278.94*	-44.44**	30.67**	8	S
MGC-15 × GP-311	15266.6	3.44**	2331.99*	88.89**	-30.73**	5	MR
MGC-32 × BML-6	14070.77	-1.65**	-341.88	-44.44**	23.89**	4	MR
MGC-32 × BML-7	14629.1	3.64**	-4561.61	88.89**	24.24**	8	S
MGC-32 × BML-14	14037.44	-2.15**	-414.11	-66.67**	-15.28**	2	R
MGC-32 × GP-170	15495.77	1.91**	-117.72*	66.67**	-42.36**	3	R
MGC-32 × GP-311	13937.44	-1.75**	1635.33*	-44.44**	-37.83**	4	MR
MGC-92 × BML-6	14683.27	1.54**	516.44	44.44**	-5.86	8	S
MGC-92 × BML-7	13291.61	-2.15**	-2114.66*	-22.22	-4.96*	6	MS
MGC-92 × BML-14	13999.94	3.04**	-283.44**	66.67**	-5.07	8	S
MGC-92 × GP-170	15362.43	1.11*	-867.72	-22.22	4.39**	3	R
MGC-92 × GP-311	13987.44	-2.55**	-2988.38*	-66.67**	-45.10*	6	MS
MGC-137 × BML-6	13766.61	-3.05**	381.44*	-44.44**	-7.61	7	MS
MGC-137 × BML-7	14049.94	2.24**	782.55**	88.89**	-29.14*	8	S
MGC-137 × BML-14	13395.78	2.44**	167.55	66.67 **	-7.97	6	MS
MGC-137 × GP-170	14421.44	-0.48	-381.88	-22.22	10.58*	4	MR
MGC-137 × GP-311	14904.1	-1.15*	2349.66	-44.44	10.77*	2	R
MGC-230 × BML-6	14912.44	2.94**	-436.05*	88.89**	10.61*	6	MS
MGC-230 × BML-7	14062.44	-2.75**	-982.36**	-22.22	-8.1	4	MR
MGC-230 × BML-14	13491.61	0.44	-49.94**	22.22	-7.04	8	S
MGC-230 × GP-170	14216.61	-1.48**	-771.44*	-44.44**	0.68*	8	S
MGC-230 × GP-311	14183.27	0.84	-2169.27	44.44**	-7.88	5	MR
MGC-237 × BML-6	13037.44	-1.05*	-1120.7*	-22.22	-0.05	8	S
MGC-237 × BML-7	14849.94	3.24**	1576.72**	88.89**	-4.23	3	R
MGC-237 × BML-14	14366.6	-1.55**	-209.11*	-44.44**	-8.1	8	S
MGC-237 × GP-170	14354.1	-0.48	-1150.2*	-44.44**	13.28*	7	MS
MGC-237 × GP-311	14570.77	-0.15	-1297.16	-22.22	-8.76*	7	MS
MGC-238 × BML-6	13883.27	1.54**	554.77*	66.67**	-3.87	8	S
MGC-238 × BML-7	14370.77	-0.15	1988.27	44.44**	12.98*	5	MR
MGC-238 × BML-14	14024.94	3.04**	- 802.44*	88.89**	-8.35*	6	MS
MGC-238 × GP-170	13891.61	-0.88	-924.7**	-22.22	0.57*	7	MS
MGC-238 × GP-311	14499.77	-3.55**	-3388.83*	-44.44**	-8.43	9	S
MGC-239 × BML-6	14395.77	1.34**	-204.38*	66.67**	-9.87*	8	S

Hybrids	Grain yield mean (kg/ha)	Specific combining ability effect		Heterosis over best check (BIO-9544)		In field (Toothpick method)	
		Disease score (1-9)	Grain yield (kg/ha)	Disease score (1-9)	Grain yield (kg/ha)	MSR mean score (1-9)	Disease reaction
Crosses							
MGC-239 × BML-7	14099.94	1.64**	113.38*	88.89**	-8.02*	7	MS
MGC-239 × BML-14	15033.27	0.84	734.94*	44.44**	-12.30*	4	MR
MGC-239 × GP-170	14345.77	-0.08	140.61	44.44**	-5.59	8	S
MGC-239 × GP-311	14045.77	-3.75**	685.33**	-44.44**	-22.43	5	MR
MGC-242 × BML-6	13749.94	0.54	-875.60**	44.44**	-2.37*	9	S
MGC-242 × BML-7	14708.27	-2.15**	1560.05	-22.22	-7.94	7	MS
MGC-242 × BML-14	15616.6	-1.95**	2807.55*	-78.89	37.14**	3	R
MGC-242 × GP-170	15383.27	1.11*	1029.38*	22.22	-6.93*	4	MR
MGC-242 × GP-311	14758.27	2.44**	-1811.33*	88.89**	-7.15*	6	MS
MGC-248 × BML-6	13954.11	-2.85**	-1061.88	-22.22	14.65*	8	S
MGC-248 × BML-7	14879.1	1.44**	-2785.05*	88.89**	-2.78	7	MS
MGC-248 × BML-14	15366.6	1.64**	2785.05**	66.67**	32.48*	4	MR
MGC-248 × GP-170	15045.77	-2.28**	-854.38*	-44.44**	-6.03**	7	MS
MGC-248 × GP-311	15370.77	2.04**	-2123.66*	88.89**	-4.61	3	R
MGC-252 × BML-6	14466.6	-2.65**	-114.38*	-66.67**	19.11*	5	MR
MGC-252 × BML-7	14004.11	-2.35**	-3407.55*	-44.44**	-5.92	8	S
MGC-252 × BML-14	15620.77	0.84	732.44*	-44.44**	-8.18	2	R
MGC-252 × GP-170	15516.6	-0.08	415.22*	-44.44**	40.67*	7	MS
MGC-252 × GP-311	15804.1	-3.15**	4179.77**	-88.89**	42.48**	3	R
MGC-254 × BML-6	14533.27	2.74 **	548.11*	77.78**	15.76*	9	S
MGC-254 × BML-7	13970.77	-1.45**	-2413.27*	77.78**	27.69*	8	S
MGC-254 × BML-14	14575.94	-1.25*	625.88*	-22.22	31.58*	3	R
MGC-254 × GP-170	15574.93	1.81**	-1771.44*	22.22	-16.08*	8	S
MGC-254 × GP-311	15804.1	-3.55**	4179.77**	-84.44**	42.48**	3	R
MGC-256 × BML-6	14174.94	2.14**	-956.88*	66.67**	9.98*	7	MS
MGC-256 × BML-7	14387.44	0.44	-2935.88*	44.44**	13.38*	7	MS
MGC-256 × BML-14	14387.44	0.64	750.05*	22.22	-12.4*	4	MR
MGC-256 × GP-170	15624.93	-1.28*	3875.60**	-82.22**	37.58**	3	R
MGC-256 × GP-311	15725.93	-3.15**	3924.77**	-80.00**	38.71**	2	R
Checks							
DHM-117	14583.27	-	-	-	-	3	R
BIO-9544	14683.27	-	-	-	-	5	MR
KAVERI-50	13508.27	-	-	-	-	9	S

R: Resistant, MR: Moderately resistant, MS: Moderately susceptible, S: Susceptible.

Table 9. Summary of *Macrophomina* stalk rot disease incidence recorded in field by toothpick method (at harvesting) using standard rating scale (score 1-9)

Parent/Cross	In field (Toothpick method)	
	MP mean score	Disease reaction
MGC-9	5	MR
MGC-137	4	MR
MGC-237	3	R
MGC-242	5	MR
MGC-248	3	R
MGC-252	4	MR
MGC-254	3	R
MGC-256	2	R
BML-6	3	R
GP-170	5	MR

Parent/Cross	In field (Toothpick method)	
	MP mean score	Disease reaction
GP-311	3	R
MGC-6 × BML-7	5	MR
MGC-6 × BML-14	4	MR
MGC-6 × GP-170	4	MR
MGC-9 × BML-6	3	R
MGC-9 × BML-14	3	R
MGC-15 ×GP-311	5	MR
MGC-32 × BML-6	4	MR
MGC-32 × BML-14	2	R
MGC-32 × GP-170	3	R
MGC-32 ×GP-311	4	MR
MGC-92 × GP-170	3	R
MGC-137 × GP-170	4	MR
MGC-137 ×GP-311	2	R
MGC-230 ×GP-311	5	MR
MGC-237 × BML-7	3	R
MGC-238 × BML-7	5	MR
MGC-239 × BML-14	4	MR
MGC-239 ×GP-311	5	MR
MGC-242 × BML-14	3	R
MGC-242 ×GP-170	4	MR
MGC-248 × BML-14	4	MR
MGC-248 ×GP-311	3	R
MGC-252 × BML-6	5	MR
MGC-252 × BML-14	3	R
MGC-252 × GP-311	3	R
MGC-254 × BML-14	3	R
MGC-254 × GP-311	3	R
MGC-256 × BML-14	4	MR
MGC-256 × GP-170	3	R
MGC-256 ×GP-311	2	R
DHM-117	3	R
BIO-9544	5	MR

MP: Macrophomina phaseolina, R: Resistant, MR: Moderately resistant, MS: Moderately susceptible, S: Susceptible.

4. CONCLUSION

On the basis of mean per se performance, gca, sca effects and heterosis, the following inbred lines *viz*., MGC-256, MGC-252, MGC-254, MGC-248 and MGC-242; and testers *viz*., GP-311, BML-14; and the hybrid crosses *viz*., MGC-252 × GP-311, MGC-254 × GP-311, MGC-256 × GP-311, MGC-256 × GP-170, MGC-242 × BML-14, MGC-248 × BML-14, MGC-254 × GP-170 and MGC-256 × BML-14 showed highest resistance owing to their good general and specific combining abilities for PFSR resistance genes. Aforesaid lines and combinations showed higher grain yield. It can be concluded from theon present investigation that five best hybrid combinations (MGC-252 × GP-311, MGC-254 × GP-311, MGC-256 × GP-311, MGC-256 × GP-170 and MGC-242 × BML-14) are superior for grain yield and PFSR disease resistance and the same may be promoted for further evaluation before releasing as commercial hybrids.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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