

Original Research Article

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Genetic Testing of Inbred Lines and Single Cross Hybrids against Fusarium Stalk Rot Caused by *Fusarium moniliforme* in Maize (*Zea mays* L.)

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ABSTRACT

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Globally, Maize (*Zea mays* L.) is a third major cereal food crop. It is a multipurpose crop with 26% of its production is used as food by human beings. Maize is known as “queen of cereals”, because of its high genetic yield potential, efficient utilization of radiant energy and wider adaptability. About 65 different phytopathogens affect the maize production in different stages of life cycle. Among which *Fusarium moniliforme* is one such soil borne pathogen causes Fusarium stalk rot (FSR) disease that ultimately reduces maize yield potential over the world. In any breeding program, screening and genetic testing of available germplasm resources against pathogens is necessary to prevent yield losses. Hence, the present research screened around 114 maize inbred lines and 45 single cross hybrids (SCHs) against FSR under artificial epiphytotic conditions. Among 114 inbreds, only four inbreds viz., CM 202, 10878, MAI-759 and MAI-766 (mean disease score of 3-4) showed moderately resistant reaction and out of 45 SCHs, only one hybrid combination i.e., MAI329 × CM202 (mean disease score was 2.60) exhibited resistance reaction against Fusarium stalk rot. Nevertheless, these resistance sources could be utilized in maize breeding programs for obtaining high yielding cultivars with resistance towards FSR disease.

Introduction

Maize (*Zea mays* L.) is a promising staple cereal crop next to wheat and rice in terms of production globally. It serves as a basic meal, livestock feed, and a raw resource for industry (Troyer, 2006). Approximately 26% of the country's maize production is eaten as food. Its high-yielding rank among the world's major crops is largely attributable to its

excellent utilization of photosynthetic solar energy and carbon dioxide fixation (C4 pathway) from the environment (Anita, 2016)). Because of its tremendous production potential, versatility and flexibility, maize is renowned as the “Queen of Cereals” (Tabassum *et al.*, 2020). Maize production also has the lowest economics when compared to other crops. The major maize-cultivating countries include the United States, China,

Brazil, India, Mexico, and Argentina. In India, the foremost maize growing states include Karnataka, Andhra Pradesh, Bihar, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, and West. However, maize monoculture and genetic homogeneity (using of similar genetic background in maize breeding programs) allow diseases to proliferate easily (Power, 1987; Liu *et al.*, 2003). Over the world, nine percent of the yield losses were estimated to be caused by diseases alone (Oerke, 2005). Maize is infected by over 65 pathogens, including fungus and bacteria that cause foliar diseases, ear rot, and stalk rots (Rahul and Singh, 2002). Fungi are the most important phytopathogens in maize cultivation, with *Fusarium* spp. being one of the most serious threats because they can infect roots, stalks, kernels, and ears (Holbert *et al.*, 1924; Munkvold, 2003). *Fusarium* stalk rot (FSR), caused by *Fusarium moniliforme*, is one of the most severe soil-borne maize diseases, affecting maize production on every continent. Rajasthan, Uttar Pradesh, Bihar, Karnataka, and Andhra Pradesh are some of the states where the disease is prevalent. Early in the season, dry and warm weather (28-30° C), followed by wet weather two to three weeks after silking, favours disease incidence. To minimize the effect of FSR, several disease management methods have been suggested, including conventional tillage, crop rotation, foliar fungicide usage, and the deployment of resistant hybrids. Planting resistant cultivars are one of these measures that can significantly prevent disease growth and is highly advised (Ward *et al.*, 1997). Breeding for resistance is a feasible and cost-effective way to manage soil-borne pathogens (Fehr, 1987). As a result, screening of maize germplasm has become a critical phase of maize breeding programs, where disease resistance selection leads to yield stability. Natural infection, on the other hand, will vary from year to year due to climatic factors and crop management practices. Henceforth, the

creation of artificial epiphytotic inoculation methods can improve the reliability of the evaluation process and allow highly susceptible genotypes to be rejected from the breeding program.

Materials and Methods

Plant material

A total of 114 maize inbred lines (table3) assembled from different parts of the globe were evaluated along with two standard susceptible checks (Hema and Nithyashree) for FSR disease reaction under field condition at the College of Agriculture, V. C. Farm, Mandya, Karnataka with two replications under Randomized Complete Block Design (RCBD) planted during summer 2016. Each entry in each replication was planted in a 2m row with spacing of 60 × 20 cm. Of the 114 inbreds screened, ten best lines (table2) that showed varied disease reactions against FSR were selected to produce all possible single cross hybrids (SCHs) in a diallel fashion during *Kharif* 2017 following recommended agronomic field practices. Artificial disease epiphytotic condition was created as screening protocol suggested by the Indian Institute of Maize Research (IIMR, New Delhi).

Creation of artificial disease epiphytotic condition

The diseased maize stalks exhibiting perfect FSR symptoms were identified and collected from all susceptible genotypes grown at the farm of College of Agriculture, V. C. Farm, Mandya. The disease-causing *Fusarium moniliforme* was isolated using the standard tissue isolation technique (IIMR), New Delhi. Pathogen isolations were done by plating surface sterilized (4 percent sodium hydrochloride) small pieces of infected tissues on potato dextrose agar (PDA) medium. Purification of cultures was made by the

hyphal tip method. The fungal hyphae were aseptically transferred to culture plates containing the sterile PDA medium to get stock culture. The culture colour of *F. moniliforme* was pinkish white (Plate1) and confirmed by the shape of microconidia. The inoculum was mass multiplied on Petri plates (Plate4). The cultures of the fungus were sub-cultured on potato dextrose agar slants and kept in the laboratory at 28±1°C for 15 days. Further, these cultures were sub-cultured once a month and used for future studies. Inoculations were made with 45-50 days old plants just after the flowering stage, in the second internode above the soil level. The suspension of *Fusarium* culture was injected diagonally using the syringe after pricking and making a 2 cm hole with the help of jabber (Plate2 and 3). Disease symptoms appeared in the inoculated plants about 20-25 days after inoculation. The disease intensity and severity were recorded following a 1 (highly resistant)-9 (highly susceptible) rating scale as described by IIMR (New Delhi) given in table1. Based on disease scoring scale, maize inbred lines and single cross hybrids were classified in to different disease reaction groups (Fig1 and 2; table5).

Results and Discussion

Due to its soil-borne infection pathway, fungicidal control of *Fusarium* stalk rot is not effective. However, to reduce disease-related losses while simultaneously boosting production to meet increasing demands, it is required to introduce a significant level of genetic resistance in breeding programs is advised and to combat yield losses caused by phytopathogens. For identification of resistance source to *Fusarium* stalk rot in maize, a total of 114 maize inbred lines were screened, and mean disease score and disease reaction was recorded by using the 1-9 disease rating scale suggested by IIMR, New Delhi during summer 2016. The results about the

mean disease score of inbred lines are presented in table3 along with the standard error of the mean (SEM±) and critical difference (CD). Similarly, all 45 single cross hybrids were screened against FSR to identify a resistant cross combination during *Kharif* 2017(table4).

Out of 114 maize inbred lines screened against *Fusarium* stalk rot, four genotypes *viz.*, CM 202, 10878, MAI-759 and MAI-766 showed moderately resistant reaction with a mean disease score of 3-4. While, 20 genotypes *viz.*, MAI-215, MAI-272, MAI-279, 18683, MAI-250, MAI-257, MAI-283, MAI-293, MAI-306, MAI-307, 33189, MAI-300, 31837, MAI-320, MAI-329, MAI-395, MAI-753, MAI-754, MAI-762, MAI-764 and MAI-767 expressed moderately susceptible reaction with mean disease score of 4-5. Fifty-two genotypes showed susceptible reaction and 38 genotypes recorded highly susceptible disease reactions with mean disease scores of 5-6 and 6-9, respectively (fig1). In the present study, a total of 114 maize genotypes were screened for identification of resistance sources to *Fusarium* stalk rot. Out of which only two inbreds *viz.*, CM202 (3.00) and MAI 766 (3.40) expressed moderately resistant reaction. Fasihi *et al.*, (2013), also reported resistance in the inbred MO17 to *Fusarium verticillioides* under field conditions. As Hooker and Draganic (1980), suggested that one resistant inbred parent is sufficient for obtaining a hybrid resistance to stalk rot. Hence the inbred line CM202 possessing moderate resistance to stalk rot would be valuable breeding stock for use as a parent for the development of resistant hybrids. Out of 114 genotypes, 15 inbreds expressed moderately susceptible reaction against *Fusarium* stalk rot with disease score of (4-5).

These results were in confirmation with Qureshi *et al.*, (2015), wherein they reported 13 inbred lines as moderately susceptible. A

total of 68 and 57 genotypes showed highly susceptible and susceptible reaction. Even Afolabi *et al.*, (2008), also reported six inbred lines with high levels of disease susceptibility. The mean disease scale ranged between 3.05 to 6.75 (table2) among ten parents used in producing all possible cross combinations while, in crosses, the range was from 2.60 to

7.25 (table4). Among 45 single crosses, only the cross MAI329 × CM202 (mean disease score was 2.60) exhibited resistance reaction against Fusarium stalk rot but the checks Nithyashree and Hema recorded susceptible reaction with mean disease scores of 5.4 and 6.2, respectively.

Table.1 Disease rating scale: (IIMR, New Delhi; Hooker, 1956)

Disease score	Disease reaction	Symptoms
1	HR	Healthy or slight discolouration at the site of inoculation
2	R	Up to 50% of the inoculated internode is discoloured
3	MR	51-75% of the inoculated internode is discoloured
4	MS	76-100% of the inoculated internode is discoloured
5	S	Less than 50% discolouration of the adjacent internode
6	HS	More than 50% discolouration of the adjacent internode
7	HS	Discolouration of three internodes
8	HS	Discolouration of four internodes
9	HS	Discolouration of five internodes and premature death of plant

Note: HR: Highly resistant, R: Resistance, MR: Moderately Resistance, MS: Moderately susceptible, S: Susceptible, HS: Highly susceptible

Table.2 List of 10 best parents used in the production of single cross hybrids

Sl. No.	Genotype	Disease score	Disease reaction
1	CM202	2.50-3.00	MR
2	MAI766	3.00	MR
3	10878	3.60	MR
4	31837	4.30	MS
5	MAI329	4.00	MS
6	MAI754	4.00	MS
7	VL109287	5.30	S
8	VL108867	7.00	HS
9	VL1043	6.30	HS
10	VL1218	6.60	HS
11	Hema (Check)	6.20	HS
12	Nithyashree (Check)	5.40	S

Table.3 Screening of inbred lines of maize for their reaction to Fusarium stalk rot

Sl.No	Inbreds	DS	DR	Sl.No	Inbreds	DS	DR	Sl.No	Inbreds	DS	DR	Sl.No	Inbreds	DS	DR
1	M-QPM-1	5.6	S	31	MAI-211	5.6	S	61	33189	4.6	MS	91	VL1043	6.3	HS
2	M-QPM-2	6.0	HS	32	MAI-212	6.3	HS	62	33189-1	6.3	HS	92	VL108867	7.0	HS
3	M-QPM-4	5.3	S	33	MAI-213	6.0	HS	63	MAI-322	5.6	S	93	VL109287	5.3	S
4	M-QPM-5	5.0	S	34	MAI-214	5.3	S	64	32785	5.6	S	94	VL109449	5.0	S
5	M-QPM-7	5.0	S	35	MAI-215	5.6	S	65	33174	6.0	HS	95	VL109452	6.0	HS
6	M-QPM-10	6.6	HS	36	MAI-223	4.6	MS	66	32554	5.0	S	96	VL121096	7.0	HS
7	M-QPM-12	6.0	HS	37	MAI-250	4.0	MS	67	MAI-332	5.6	S	97	VL1218	6.6	HS
8	MAI-13	5.3	S	38	10250	5.3	S	68	MAI-333	5.3	S	98	VL1236	6.6	HS
9	MAI-14	6.0	HS	39	MAI-252	5.0	S	69	MAI-364	6.0	HS	99	CML-4	7.0	HS
10	MAI-21	5.3	S	40	MAI-257	4.3	MS	70	MAI-386	5.6	S	100	CML-9	6.0	HS
11	M-QPM-22	5.3	S	41	MAI-266	5.3	S	71	MAI-391	5.0	S	101	CML-16	5.0	S
12	MAI-28	5.3	S	42	MAI-267	5.3	S	72	MAI-395	4.6	MS	102	CML-17	5.6	S
13	M-QPM-33	6.0	HS	43	MAI-268	5.3	S	73	MAI-702	6.3	HS	103	CML-20	5.0	S
14	M-QPM-35	6.0	HS	44	MAI-272	4.6	MS	74	MAI-727	5.3	S	104	CML-21	5.0	S
15	M-QPM-38	6.0	HS	45	MAI-279	4.6	MS	75	MAI-740	5.0	S	105	CML-22	6.0	HS
16	M-QPM-39	5.6	S	46	MAI-282	5.6	S	76	MAI-746	5.6	S	106	CML-23	5.3	S
17	MAI-40	6.6	HS	47	MAI-283	4.0	MS	77	MAI-751	5.6	S	107	CML-31	5.0	S
18	10269	6.0	HS	48	MAI-285	5.0	S	78	MAI-753	4.0	MS	108	CML-35	5.6	S
19	MAI-103	5.6	S	49	MAI-292	5.6	S	79	MAI-754	4.0	MS	109	CML-37	7.0	HS
20	10878	3.6	MR	50	MAI-293	4.0	MS	80	MAI-758	5.3	S	110	CML-39	6.3	HS
21	MAI-134	6.0	HS	51	MAI-306	4.3	MS	81	MAI-759	3.6	MR	111	CML-153	6.0	HS
22	MAI-135	5.3	S	52	MAI-307	4.6	MS	82	MAI-762	4.6	MS	112	CML-137	7.3	HS
23	MAI-137	6.3	HS	53	MAI-310	5.3	S	83	MAI-764	4.6	MS	113	CML-139	6.0	HS
24	MAI-150	6.0	HS	54	31810	5.3	S	84	MAI-766	3.0	MR	114	CM202	3.0	MR
25	MAI-155	5.6	S	55	31837	4.3	MS	85	MAI-767	4.6	MS		S Em±	0.48	
26	MAI-175	5.6	S	56	MAI-320	4.3	MS	86	VL05550	6.3	HS		CD@5%	1.34	
27	MAI-182	6.3	HS	57	32427	5.0	S	87	VL073318	5.0	S	Total			
28	18683	4.3	MS	58	32645	5.0	S	88	VL1012849	5.3	S				
29	MAI-202	6.3	HS	59	32810	5.0	S	89	VL1018792	7.0	HS	MR	4	S	38
30	MAI-206	6.6	HS	60	MAI-329	4.0	MS	90	VL1033	7.0	HS	MS	19	HS	54

Note: DS: Disease Score, DR: Disease reaction, HR: Highly resistant, R: Resistance, MR: Moderately Resistance, MS: Moderately susceptible, S: Susceptible, HS: Highly susceptible

Table.4 Screening of single cross hybrids of maize for their reaction to Fusarium stalk rot

Sl.No	SCHs	DS	DR	Sl.No	SCHs	DS	DR	Sl.No	SCHs	DS	DR
1	MAI-766×CM202	5.10	S	19	VL109287×VL108867	5.00	S	37	VL1043×10878	6.80	HS
2	MAI-329×CM202	2.60	R	20	VL1218×VL1043	4.38	MS	38	VL1218×10878	5.50	S
3	MAI-754×CM202	4.25	MS	21	10878×MAI-329	6.00	HS	39	VL109287×10878	6.50	HS
4	10878×CM202	4.38	MS	22	31837×MAI-329	6.40	HS	40	VL108867×31837	7.00	HS
5	31837×CM202	5.45	S	23	VL108867×MAI-329	5.80	S	41	VL1043×31837	6.25	HS
6	VL108867×CM202	4.40	MS	24	VL1043×MAI-329	5.60	S	42	VL1218×31837	6.00	HS
7	VL1043×CM202	5.85	S	25	VL1218×MAI-329	5.75	S	43	VL109287×31837	6.63	HS
8	VL1218×CM202	4.60	MS	26	VL109287×MAI-329	6.25	HS	44	VL1043×VL108867	5.30	S
9	VL109287×CM202	6.10	HS	27	10878×MAI-754	7.25	HS	45	VL1218×VL108867	4.40	MS
10	MAI-329×MAI-766	5.25	S	28	31837×MAI-754	6.20	HS	46	Nithyashree (Check)	5.40	S
11	MAI-754×MAI-766	5.85	S	29	VL108867×MAI-754	5.63	S	47	Hema (check)	6.20	HS
12	10878×MAI-766	6.10	HS	30	VL1043×MAI-754	6.38	HS		Grand Mean	5.65	
13	31837×MAI-766	6.50	HS	31	VL1218×MAI-745	6.25	HS		Range	2.6- 7.25	
14	VL108867×MAI-766	6.38	HS	32	VL109287×MAI-754	5.63	S		SEm±	0.32	
15	VL1043×MAI-766	5.60	S	33	31837×10878	6.30	HS		C.D. @ 0.05	0.64	
16	VL1218×MAI-766	5.63	S	34	VL108867×10878	6.63	HS		C.D. @ 0.01	0.86	
17	VL109287×MAI-766	6.22	HS	35	VL109287×VL1043	5.25	S				
18	MAI-754×MAI-329	4.75	MS	36	VL109287×VL1218	4.10	MS				

Note: DS: Disease Score, DR: Disease reaction, HR: Highly resistant, R: Resistance, MR: Moderately Resistance, MS: Moderately susceptible, S: Susceptible, HS: Highly susceptible

Table.5 Grouping of maize inbreds and single cross hybrids based on Fusarium stalk rot disease reaction

Sl.no.	DR	Name of the Inbreds	Name of the SCHs	DS	Total count
1.	HR	-	-	1	0
2.	R	-	MAI-329×CM202	2-2.9	1
3.	MR	MAI-766,CM202,10878,MAI-759	-	3-3.9	4
4.	MS	MAI-250,283,293,329,753,754,257,306,320,223,272,279,307,395,762,764,767,18683,31837,33189,	MAI-754×CM202, 10878×CM202, VL108867×CM202, VL1218×CM202, MAI-754×MAI-329, VL1218×VL1043, VL109287×VL1218, VL1218×VL108867	4-4.9	28
5.	S	M-QPM-5,M-QPM-7,MAI-252,285,391,740,32427,32654,32810,32554,VL073318,VL109449,CML-16,20,21,31,M-QPM-4,MAI-13,21,M-QPM-22,MAI-28,135,214,10250,MAI-266,267,268,310,333,727,31810,VL1012849,VL109287,CML-23,M-QPM-1,M-QPM-39,MAI-103,155,175,211,215,282,292,322,32785,MAI-332,746,751,CML-17,CML-35	MAI-766 ×CM202, 31837×CM202, VL1043× CM202, MAI-329×MAI-766, MAI-754×MAI-766, VL1218×MAI-766, VL109287×VL108867, VL108867×MAI-329, VL1043×MAI-329, VL1218×MAI-329, VL108867×MAI-754, VL109287×MAI-754, VL109287×VL1043, VL1218×10878, VL1043×VL108867, VL1043×MAI-766	5-5.9	51
6.	HS	M-QPM-2,12,33,38,10,MAI-14,134,150,213,364,137,182,202,212,702,40,206,CML-4,37,137,39,9,22,153,139,VL109452,VL05550,VL1043,VL1218,VL1236,VL1018792,VL1033,VL108867,VL121096,33189-1,33174,10269	VL109287×CM202,10878×MAI-766, 31837×MAI-766, VL108867×MAI-766, VL109287×MAI-766, 10878×MAI-329, 31837×MAI-329, VL109287×MAI-329, 10878×MAI-754, 31837×MAI-754, VL1043×MAI-754, VL1218×MAI-745, 31837×10878, VL108867×10878, VL1043×10878, VL109287×10878, VL108867×31837, VL1043×31837, VL1218×31837, VL109287×31837	6-9	75

Note: DS: Disease Score, DR: Disease reaction, HR: Highly resistant, R: Resistance, MR: Moderately Resistance, MS: Moderately susceptible, S: Susceptible, HS: Highly susceptible

Plate.1 Culture colour of *Fusarium moniliforme*



Plate.2 and 3 Syringe method of inoculation



Plate.4 Mass multiplication of *Fusarium* culture on Petri plates



Fig 1. The maize inbred lines showed varied disease reactions against *Fusarium* stalk rot (FSR) screened under artificial epiphytotic conditions

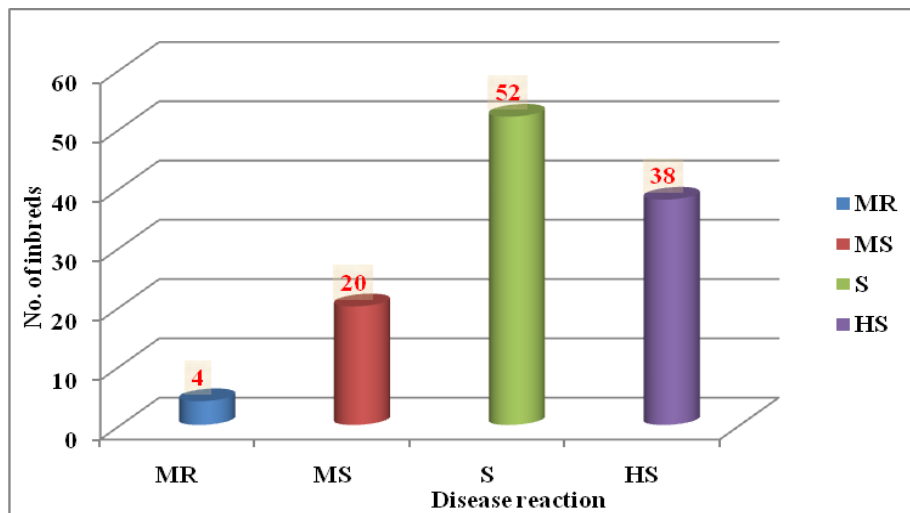
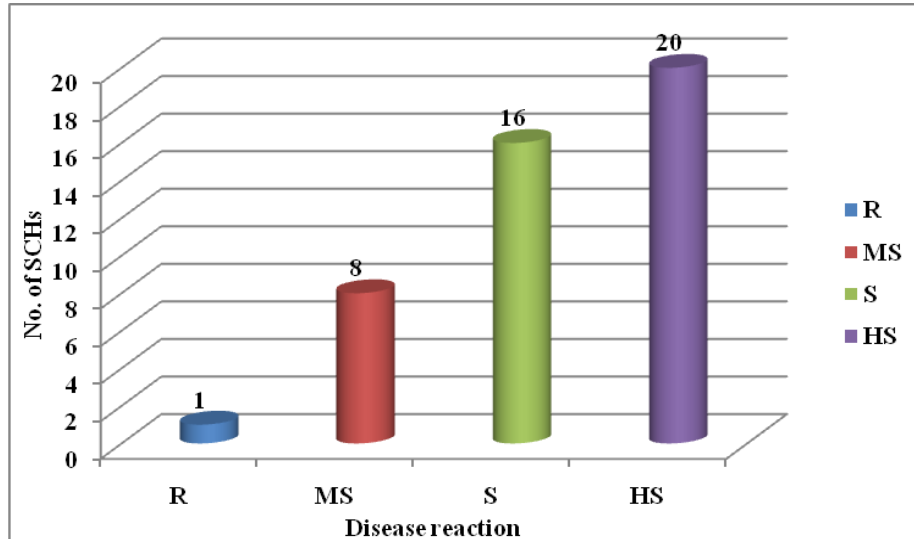


Fig 2. Screening of single cross hybrids (SCH) against *Fusarium* stalk rot (FSR) under artificial epiphytotic conditions



Therefore, the cross MAI329 × CM202 with high grain yield and resistance to *Fusarium* stalk rot needs to be evaluated in extensive yield trials for release as a new hybrid. Of the 45 SCHs, eight single crosses *viz.*, MAI-754×CM202, 10878×CM202, VL108867×CM202, VL1218×CM202, MAI-754×MAI-329, VL1218×VL1043, VL109287 ×VL1218, VL1218×VL108867 showed moderately susceptible disease reaction towards FSR with a mean disease score range of 4-4.9. While 16 cross combinations showed susceptible reaction and 20 single crosses exhibited highly susceptible reaction against FSR disease with mean disease scores of 5-5.9 and 6-9, respectively (fig2). An earlier report by Ledencan *et al.*, (2003), suggests that testing against FSR needs to be screened in both inbred lines and hybrids. Based on the mean disease rating scale maize inbred lines and single cross hybrids were classified into different disease reaction groups (table5). Screening and genetic evaluation of germplasm against biotic stresses is an integral part of any breeding program. Since *Fusarium moniliforme* is a soil-borne pathogen, breeding and deployment of resistant cultivars having good agronomic performance are

recommended over other means of combating yield losses by the pathogen around the globe.

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