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Screening Technique for Identification of Resistant Genotypes against Post Flowering Stalk Rot Complex caused by *Macrophomina phaseolina* in Maize (*Zea mays* L.)

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A B S T R A C T

In order to screen maize genotypes for resistance to post-flowering stalk rot (PFSR) complex caused by *Macrophomina phaseolina* under field conditions, toothpick method was used for creating artificial epiphytotics. In this study, 98 maize genotypes (20 parents (15 Lines × 5 testers), 75 Single Cross Hybrids (SCHs) and three standard checks) were screened in field by toothpick method of inoculation. The field screening of maize genotypes by the standard toothpick method which needs about 40 days (only at harvesting stage) for expression of plant drying symptoms due to PFSR and data are possible to record only at the time of crop harvesting. Screening is done by using 1-9 rating scale of PFSR for scoring disease severity in-vivo condition. All these maize inbred lines were screened in field by toothpick method of inoculation at Maize Research Centre, Agricultural Research Institute, Rajendranagar, Hyderabad. As a result, most of the genotypes were exhibited disease reaction varying from resistant (score-2) to moderately resistant (score-5) against *M. phaseolina*. 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.

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. The global maize production is about 1.09 billion metric tonnes from 153.0 million hectares. 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 (INDIASTAT, 2016-17).

In India yield lag is one of the major constraints that hinder maize production. Apart from pest and diseases, fungal diseases like, post flowering stalk rots (PFSR) poses a major threat to the productivity of maize (Sharma *et al.,* 1993). PFSR is a complex disease of maize, 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 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% (Desai *et al.,* 1991), 13.2 to 39.5% (Payak and Sharma 1985), 25.0 to 32.0% (Kumar *et al.,* 1998), 10.0 to 42.0% (Harlapur *et al.,* 2002), 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% (AICRP, 2014).

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 (Nagy and Cabulea, 1996). 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 (Shekhar *et al.,* 2010, Hooda *et al.,* 2012) and abroad (Clark and Foley 1985). 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 (Anon. 1983, 2012). But this method requires longer time for disease development and rotting symptoms in the inoculated stalks become prominent only at harvesting stage. But now, in the current investigation tooth pick method is used in the field.

Materials and Methods

Disease sick plot development is crucial in successes of breeding for resistance to PFSR. Screening reinforced with artificial inoculation using tooth pick method is effective in supplementing the disease sick plot technique of screening against PFSR. The methodology fallowed is suitable for screening against a multi-pathogen disease complex (Shankar Lingam and Venkatesh, 2005). Post-flowering stalk rot of maize occur in both the growing season's *viz., kharif* (rainy) and *Rabi* (winter) at Maize Research Centre, Agriculture Research Institute, Rajendranagar, Hyderabad, a disease sick plot was developed by incorporating infected stubbles of all the casual organisms of PFSR over a period of more than three decades.

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. 100 ml (peptone 1g, honey 5ml and distilled water 94ml) sterilized (20 minutes) and cooled honey peptone medium was poured under aseptic condition into a sterilized, wide mouthed bottle with screw cap, containing toothpicks.

Then from stock culture, two loops of mycelial 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.

Seeds of 20 maize genotypes were collected from the Maize Research Centre (MRC), Hyderabad. The genotypes were crossed to generate 75 SCHs during Kharif, 2019 in L × T mating design (Table 1) and these 20 parents (15 Lines and 5 Testers) and 75 SCHs along with three standard checks, a total of 98 genotypes were subjected to evaluated by raising the crop in disease sick plot accompanied by toothpick inoculation during Rabi, 2019-20, at the same institute.

Inoculation of the plants of 45-50 days old was done just after flowering by toothpick method (Anon. 1983 and 2012. 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). 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 Payak and Sharma (1983) was followed for scoring and scale has been unequally distributed into four categories of disease severity (Table 2), viz., resistant (R), moderately resistant (MR), moderately susceptible (MS) and susceptible reaction (S).

All data on the disease severity generated from the experiments conducted in field was assessed at the end.

Results and Discussion

Screening of maize genotypes in field (Table 3): 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. Whereas, 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 only one tester, BML-7 was found susceptible.

Table1 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		
1	MGC-6	50	MGC-137 × GP-311
2	MGC-9	51	MGC-230 × BML-6
3	MGC-15	52	MGC-230 × BML-7
4	MGC-32	53	MGC-230 × BML-14
5	MGC-92	54	MGC-230 × GP-170
6	MGC-137	55	MGC-230 × GP-311
7	MGC-230	56	MGC-237 × BML-6
8	MGC-237	57	MGC-237 × BML-7
9	MGC-238	58	MGC-237 × BML-14
10	MGC-239	59	MGC-237 × GP-170
11	MGC-242	60	MGC-237 × GP-311
12	MGC-248	61	MGC-238 × BML-6
13	MGC-252	62	MGC-238 × BML-7
14	MGC-254	63	MGC-238 × BML-14
15	MGC-256	64	MGC-238 × GP-170
	Testers	65	MGC-238 × GP-311
16	BML-6	66	MGC-239 × BML-6
17	BML-7	67	MGC-239 × BML-7
18	BML-14	68	MGC-239 × BML-14
19	GP-170	69	MGC-239 × GP-170
20	GP-311	70	MGC-239 × GP-311
	Crosses	71	MGC-242 × BML-6
21	MGC-6 × BML-6	72	MGC-242 × BML-7
22	MGC-6 × BML-7	73	MGC-242 × BML-14
23	MGC-6 × BML-14	74	MGC-242 × GP-170
24	MGC-6 × GP-170	75	MGC-242 × GP-311
25	MGC-6 × GP-311	76	MGC-248 × BML-6
26	MGC-9 × BML-6	77	MGC-248 × BML-7
27	MGC-9 × BML-7	78	MGC-248 × BML-14
28	MGC-9 × BML-14	79	MGC-248 × GP-170
29	MGC-9 × GP-170	80	MGC-248 × GP-311
30	MGC-9 × GP-311	81	MGC-252 × BML-6
31	MGC-15 × BML-6	82	MGC-252 × BML-7
32	MGC-15 × BML-7	83	MGC-252 × BML-14
33	MGC-15 × BML-14	84	MGC-252 × BML-170
34	MGC-15 × GP-170	85	MGC-252 × BML-311
35	MGC-15 × GP-311	86	MGC-254 × BML-6
36	MGC-32 × BML-6	87	MGC-254 × BML-7
37	MGC-32 × BML-7	88	MGC-254 × BML-14
38	MGC-32 × BML-14	89	MGC-254 × GP-170
39	MGC-32 × GP-170	90	MGC-254 × GP-311
40	MGC-32 × GP-311	91	MGC-256 × BML-6
41	MGC-92 × BML-6	92	MGC-256 × BML-7
42	MGC-92 × BML-7	93	MGC-256 × BML-14
43	MGC-92 × BML-14	94	MGC-256 × GP-170
44	MGC-92 × GP-170	95	MGC-256 × GP-311
45	MGC-92 × GP-311		**Checks**
46	MGC-137 × BML-6	96	DHM-117
47	MGC-137 × BML-7	97	BIO-9544
48	MGC-137 × BML-14	98	KAVERI-50
49	MGC-137 × GP-170		

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

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	

Table.3 Disease incidence of *Macrophomina* stalk rot 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
Lines		
MGC-6	7	MS
MGC-9	5	MR
MGC-15	8	S
MGC-32	7	MS
MGC-92	6	MS
MGC-137	4	MR
MGC-230	8	S
MGC-237	3	R
MGC-238	6	MS
MGC-239	7	MS
MGC-242	5	MR
MGC-248	3	R
MGC-252	4	MR
MGC-254	3	R
MGC-256	2	R

Testers		
BML-6	3	R
BML-7	8	S
BML-14	7	MS
GP-170	5	MR
GP-311	3	R
Crosses		
MGC-6 × BML-6	7	MS
MGC-6 × BML-7	5	MR
MGC-6 × BML-14	4	MR
MGC-6 × GP-170	4	MR
MGC-6 × GP-311	6	MS
MGC-9 × BML-6	3	R
MGC-9 × BML-7	7	MS
MGC-9 × BML-14	3	R
MGC-9 × GP-170	8	S
MGC-9 × GP-311	7	MS
MGC-15 × BML-6	7	MS
MGC-15 × BML-7	8	S
MGC-15 × BML-14	8	S
MGC-15 × GP-170	8	S
MGC-15 × GP-311	5	MR
MGC-32 × BML-6	4	MR
MGC-32 × BML-7	8	S
MGC-32 × BML-14	2	R
MGC-32 × GP-170	3	R
MGC-32 × GP-311	4	MR
MGC-92 × BML-6	8	S
MGC-92 × BML-7	6	MS
MGC-92 × BML-14	8	S
MGC-92 × GP-170	3	R
MGC-92 × GP-311	6	MS
MGC-137 × BML-6	7	MS
MGC-137 × BML-7	8	S
MGC-137 × BML-14	6	MS
MGC-137 × GP-170	4	MR
MGC-137 × GP-311	2	R
MGC-230 × BML-6	6	MS
MGC-230 × BML-7	4	MR
MGC-230 × BML-14	8	S
MGC-230 × GP-170	8	S
MGC-230 × GP-311	5	MR
MGC-237 × BML-6	8	S
MGC-237 × BML-7	3	R
MGC-237 × BML-14	8	S

MGC-237 × GP-170	7	MS
MGC-237 × GP-311	7	MS
MGC-238 × BML-6	8	S
MGC-238 × BML-7	5	MR
MGC-238 × BML-14	6	MS
MGC-238 × GP-170	7	MS
MGC-238 × GP-311	9	S
MGC-239 × BML-6	8	S
MGC-239 × BML-7	7	MS
MGC-239 × BML-14	4	MR
MGC-239 × GP-170	8	S
MGC-239 × GP-311	5	MR
MGC-242 × BML-6	9	S
MGC-242 × BML-7	7	MS
MGC-242 × BML-14	3	R
MGC-242 × GP-170	4	MR
MGC-242 × GP-311	6	MS
MGC-248 × BML-6	8	S
MGC-248 × BML-7	7	MS
MGC-248 × BML-14	4	MR
MGC-248 × GP-170	7	MS
MGC-248 × GP-311	3	R
MGC-252 × BML-6	5	MR
MGC-252 × BML-7	8	S
MGC-252 × BML-14	2	R
MGC-252 × GP-170	7	MS
MGC-252 × GP-311	3	R
MGC-254 × BML-6	9	S
MGC-254 × BML-7	8	S
MGC-254 × BML-14	3	R
MGC-254 × GP-170	8	S
MGC-254 × GP-311	3	R
MGC-256 × BML-6	7	MS
MGC-256 × BML-7	7	MS
MGC-256 × BML-14	4	MR
MGC-256 × GP-170	3	R
MGC-256 × GP-311	2	R
Checks		
DHM-117	3	R
BIO-9544	5	MR
KAVERI-50	9	S

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

Table.4 Summary of post-flowering stalk rot (PFSR) disease reaction observed in field condition

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
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.

Whereas, 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.

The disease severity was recorded in the field by using a scale (1-9 cm) of Payak and Sharma (1983). 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*. Disease severity data obtained from the field was summarized based on their average disease reaction presented in the (Table 4). In contrast, the currently followed inoculation procedure developed by Payak and Sharma (1983) 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 is recorded in the field by observing the disease symptoms on the whole/individual plant. Hence, for recording data in field, Payak and Sharma (1983) prescribed 1-9 scale has been unequally distributed into four categories of disease severity, viz., resistant (1 to 3), moderately resistant (4 to 5), moderately susceptible (6 to 7) and susceptible reaction (8 to 9). This scale could be efficiently used for recording severity of PFSR *in-vivo* within 40 days following toothpick method of inoculations. The artificial epiphytotic condition for PFSR disease is created by inoculating the plants in the field just after flowering mainly by toothpick method of inoculation. But this method requires longer time for disease development and rotting symptoms in the inoculated stalks become prominent only at harvesting stage. 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.

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