Plant Archives Vol. 24, No. 2, 2024 pp. 1873-1878 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.no.2.268

SCREENING OF MAIZE INBRED LINES AGAINST STALK ROT (*MACROPHOMINA PHASEOLINA* AND *FUSARIUM VERTICILLOIDES*) USING TOOTHPICK METHOD OF INOCULATION

Samantha Azmeera[1*], Y. Hari[2], D. Bhadru[3] and B. Mallaiah[4]

[1]Department of Genetics and Plant Breeding, P.J.T.S.A.U., Rajendranagar, Hyderabad, India.
[2]Department of Biotechnology, R.A.R.S., Warangal, Telangana, India.
[3]Department of Genetics and Plant Breeding, MRC, ARI Campus, Rajendranagar, Hyderabad, India.
[4]Department of Plant Pathology, M.R.C., A.R.I. Campus, Rajendranagar, Hyderabad, India.
*Corresponding author E-mail : samanthaazmeera98937@gmail.com

(Date of Receiving-01-06-2024; Date of Acceptance-07-08-2024)

ABSTRACT

Maize is considered the queen of cereals because of its tremendous producing capacity. However, throughout its life cycle, the crop faces several problems, one of which is diseases. One of the most serious of these diseases is Post flowering stalk rot (PFSR) and it is complex of fungi and bacteria. Finding the resistant inbred lines is therefore extremely important. In this study, 40 maize inbred lines along with a resistant check and a susceptible check were screened against *Macrophomina phaseolina* and *Fusarium verticilloides* using toothpick method of inoculation. The lines MRC L 6, MRC L 13, MRC L 29, MRC L 33 observed to be resistant to both the pathogens. The lines MRC L 1, MRC L 20, MRC L 24, MRC L 25, MRC L 26, MRC L 27, MRC L 30, MRC L 31 has shown the reaction of moderate resistance towards both the diseases. The resistant lines found in this study can be used as parents for producing hybrids with good yield and disease resistance to PFSR complex.

Key words : *Macrophomina phaseolina*, *Fusarium verticilloides*, Resistance, Susceptible, Toothpick method of inoculation.

Introduction

Maize (Zea *mays* L.) is third most important cereal crop after rice and wheat and it is known as the queen of cereals because it has the highest genetic yield potential among the cereals. In addition to staple food for human being and quality feed for animals, maize serves as a basic raw material as an ingredient in thousands of industrial products (APEDA). Globally Maize is cultivated in an area of 193.7 million hectares with a production of 1147.7 million tonnes and productivity of 5.75 tonnes per hectare (FAO STAT, 2020). In India, maize is grown in an area of 9.89million hectares with a production of 31.65 million tonnes and productivity of 31.99 quintals per hectare (INDIA STAT, 2020-2021).

Despite maize being a productive crop, its journey to high yields is riddled with challenges throughout its growth cycle. Among biotic stresses diseases are one of the major constraints. Estimated annual loss due to major diseases in maize in India is about 13.2 to 39.5% (Payak and Sharma, 1985). Among the major diseases Post flowering stalk rot (PFSR) complex is one of the most serious, destructive and widespread groups of diseases in maize (Khokar *et al.*, 2014). It is a silent thief, causing significant yield losses and posing a challenge to farmers. In India, eight fungi and three bacteria were reported to cause stalk rots (Raju *et al.*, 1976).

PFSR is a complicated illness as several fungi, bacteria, and nematodes are involved. In India, the disease is prevailing in most of the maize growing areas, notably in rainfed areas *viz.*, Jammu and Kashmir, Punjab, Haryana, New Delhi, Rajasthan, Madhya Pradesh, Uttar Pradesh, Bihar, West Bengal, Andhra Pradesh, Telangana, Tamil Nadu and Karnataka, where water stress occurs after flowering stage of the crop (Khokhar *et al*., 2014). Among allthe pathogens of PFSR, Fusarium stalk rot, charcoal rot and late wilt are most prevalent and destructive (Khokhar *et al*., 2014).

Macrophomina phaseolina shows Seed borne nature and has been reported by many workers (Sandra *et al*., 2008). It is reported to cause considerable yield loss in grain ranging from 25-32.2 % and deterioration in fodder quality (Krishna *et al*., 2013). *F. verticillioides* continues to wreak havoc on crops, especially maize. This fungus is particularly troublesome because it can in survive harsh conditions, in crop residue within the soil or on the soil surface (Nyvall and Kommedahl, 1970). *Fusarium verticillioides* is more common in regions with hot and dry growing conditions (Doohan *et al*., 2003), especially before or during pollination (Pascal *et al*., 2002).

In India, Shekhar *et al*. (2010), Hooda *et al*. (2012) and abroad (Clark and Foley, 1985), screening of existing genotypes against the pathogens was carried out under artificial epiphytotic conditions in order to generate disease resistance cultivars. This resulted in a collection of stalk rot resistant germplasm. A resistant genotype of maize was discovered in the study on charcoal rot using the toothpick technique of inoculation (Bhaskar *et al*., 2023). Few resistant maize genotypes were also found by Sravya *et al*. (2023). The present study contributes to the identification of genotypes resistant to both diseases. These lines may then be utilized as parental lines to create hybrids that are resistant to the PFSR complex.

Materials and Methods

To identify the resistant maize inbred lines to post flowering stalk rot of maize. During *kharif* 2022 and *Rabi* 2022-23, a total of 42 maize genotypes including a resistant check JCY 2-7 and a susceptible check Kaveri 50 (Table 1) were assessed in randomized block design with two replications. Every entry was sown in two rows, each measuring four meters in length with spacing of 60 × 20 cm. The experiment was conducted at Maize Research Station, Rajendranagar, Hyderabad situated at $17^{o}19'$ North Latitude and $79^{o}23'$ East Longitude and 542.6 meter altitude.

The inbred lines were evaluated for charcoal rot and fusarium stalk rot by inoculating the inbred lines with *Macrophomina phaseolina* and *Fusarium verticilloides* pathogen cultures by using toothpick method of inoculation. Sterile, wide-mouthed bottles with pathogen culture and toothpicks placed inside it and closed with screw lid were collected from pathology laboratory, Maize Research Station, Rajendranagar, Hyderabad. These fungus-covered toothpicks were used for the inoculation in the field. The data of the two seasons was also subjected to statistical pooled analysis (Table 4).

Characterization of fungal isolates

Identification of fungal isolate *M. phaseolina* was confirmed by examining the cultures on the characters like sooty black colour of the culture. Similarly, *F. verticilloides* was confirmed based on the features like pinkish white fungal colony (Hooda *et al*., 2018).

Pathogen inoculation

The most suitable plant stage for inoculation is between tasselling and pollination. The lower internode (second or third) above soil level was chosen. One jabber was made prior to inoculation by driving or fixing a toothpick-sized nail into a wooden handle. Next, the round toothpick bearing inoculums was inserted into the hole, effectively sealing it to avert the inoculums from drying.

During the harvesting stage, the disease response was measured on a scale of 1 to 9 to determine the severity of the charcoal rot disease. The most accurate way to assess the amount and severity of stalk rot is to split the stalk open and look for it. The scale 1-9 suggested by Payak and Sharma (1983) and Hooda *et al*. (2018) is used for disease scoring (Table 2).

Table 1 : List of inbred lines screened in this study.

S. no.	Maize inbred lines	S. no.	Maize inbred lines
1	MRC L1	22	MRC L 22
2	MRC L 2	23	MRC L 23
3	MRC L 3	24	MRC L 24
4	MRC L 4	25	MRC L 25
5	MRC L 5	26	MRC L 26
6	MRC L 6	27	MRC L 27
7	MRC L 7	28	MRC L 28
8	MRC L 8	29	MRC L 29
9	MRC L 9	30	MRC L 30
10	MRC L 10	31	MRC L 31
11	MRC L 11	32	MRC L 32
12	MRC L 12	33	MRC L 33
13	MRC L 13	34	MRC L 34
14	MRC L 14	35	MRC L 35
15	MRC L 15	36	MRC L 36
16	MRC L 16	37	MRC L 37
17	MRC L 17	38	MRC L 38
18	MRC L 18	39	MRC L 39
19	MRC L 19	40	MRC L 40
20	MRC L 20	41	JCY 2-7
21	MRC L 21	42	Kaveri 50

Table 2 : Disease rating scale.

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 internodes.	
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.	

Results and Discussion

The two season's data is subjected to the statistical analysis. The results from the two seasons pooled ANOVA (Table 3) indicated that both environmental conditions and the treatments used in this study had a significant effect on the incidence or severity of *Macrophomina* and *Fusarium*.

The mean plant data from each replication for a single season are used to calculate the disease score in this case, and the same procedure is applied for the second season. *Fusarium verticilloides* and *Macrophomina phaseolina* are the two diseases that adhere to this.

The disease score is given in the table for both the pathogens and for both the seasons. The mean of disease score is taken which included two seasons for corresponding pathogens *Macrophomina phaseolina* and *Fusarium verticilloides* (Table 4).

Plates 1, 2, 3 are representing resistance, moderately resistant, moderately susceptible reactions for charcoal rot disease.

Plates 4, 5, 6 are representing resistance, moderately resistant, moderately susceptible reactions for fusarium stalk rot disease.

The mean values of disease score from two seasons for the pathogen *Macrophomina phaseolina*, which is the causal organism of the disease charcoal rot showed that the lines MRC L 6, MRC L 10, MRC L 13, MRC L23, MRC L 29, MRC L 33 has demonstrated the resistance reaction towards the *Macrophomina phaseolina* pathogen, the lines MRC L 1, MRC L 3, MRC L 4, MRC L 8, MRC L 12, MRC L 20, MRC L 24, MRC L 25, MRC L 26, MRC L 27, MRC L 30, MRC L 31, MRC L 34, MRC L 35, MRC L 36, MRC L 37, MRC L 39 has shown the disease reaction of moderately resistant towards the pathogen *Macrophomina phaseolina* and the inbred lines MRC L 2, MRC L5, MRC L 7, MRC L 9, MRC L 11, MRC L 14, MRC L 16, MRC L 17, MRC L 18, MRC L 19, MRC L 21, MRC L 22, MRC L 28, MRC L 32, MRC L 15, MRC L 38, MRC L 40 has exhibited the disease reaction of moderately susceptible to susceptible. The check JCY 2-7 has shown resistant reaction and kaveri 50 has shown susceptible reaction.

Table 3 : ANOVA (Pooled).

Source of variation	*Macrophomina phaseolina*		*Fusarium verticilloides*	
	df	Mean squares	df	Mean squares
Environments	1	4.60024***	1	9.11869***
Treatments	41	6.19845***	41	7.29296***
Pooled error	82	0.24556	82	0.20971
Joint pooled error	123	0.22834	123	0.21395

The following findings were obtained using the mean disease score average over two seasons for the pathogen *Fusarium verticilloides*, which is the cause of *Fusarium* stalk rot. The lines MRC L 6, MRC L 12, MRC L 13, MRC L 14, MRC L 17, MRC L 29, MRC L 33 has shown the disease reaction of resistance towards the pathogen *Fusarium verticilloides*, the lines MRC L 1, MRC L 5, MRC L 7, MRC L 9, MRC L19, MRC L 20, MRC L 24,

Table 4 : Inbred lines disease score pertaining to two seasons and two pathogens.

Inbred lines	*Macrophomina phaseolina*				*Fusarium verticilloides*			
	Kharif	***Rabi***	**Mean**	**Disease score**	***Kharif***	***Rabi***	**Mean**	**Disease score**
MRC L 1	3.4	3	3.2	MR	5	4	4.5	MR
MRC L 2	6	5	5.5	MS	6.2	6	6.1	MS
MRC L 3	5	4.8	4.9	MR	6	5.5	5.75	MS
MRC L 4	5.2	5	5.1	MS	6	4.5	5.25	MS
MRC L 5	6	5	5.5	MS	5	4	4.5	MR
MRC L 6	3	3	3	R	3	2.8	2.9	R
MRC L 7	5.5	6	5.75	MS	5	4.8	4.9	MR
MRC L 8	5	5.2	5.1	MS	6	5	5.5	MS
MRC L 9	6	5	5.5	MS	5	5	5	MR
MRC L10	3	2.8	2.9	R	5.2	5	5.1	MS
MRC L 11	5.6	5.4	5.5	MS	5.6	6	5.8	MS
MRC L 12	5.2	5	5.1	MS	3	3	3	R
MRC L 13	2.8	3.2	3	R	3	2.8	2.9	R
MRC L 14	5.4	5	5.2	MS	3.2	3	3.1	R
MRC L 15	7.1	7.1	7.1	S	6	5.6	5.8	MS
MRC L 16	6	5	5.5	MS	7.5	6.6	7.1	S
MRC L 17	6	5	5.5	MS	2.8	2.2	2.5	R
MRC L 18	5.4	6	5.7	MS	5.2	5.8	5.5	MS
MRC L 19	5.5	5	5.25	MS	5	4.6	4.8	MR
MRC L 20	4	3.6	3.8	MR	5	4	4.5	MR
MRC L 21	5.8	5.2	5.5	MS	6	5.4	5.7	MS
MRC L 22	6.5	6	6.25	MS	6.8	7	6.9	MS
MRC L 23	3	2.8	2.9	R	7.1	7.1	7.1	S
MRC L 24	4.5	4	4.25	MR	5	4.6	4.8	MR
MRC L 25	5	4.3	4.65	MR	5.5	5	5.25	MS
MRC L 26	4	4.5	4.25	MR	5	4	4.5	MR
MRC L 27	4	3.5	3.75	MR	5	4	4.5	MR
MRC L 28	5.5	4.6	5.05	MR	4.5	4	4.25	MR
MRC L 29	3	3	3	R	2.5	2.8	2.65	R
MRC L 30	5.5	4.8	5.15	MS	4.8	4	4.4	MR
MRC L 31	3.5	3	3.25	MR	4.6	3.5	4.05	MR
MRC L 32	6.5	5.8	6.15	MS	7.2	6.9	7.1	S
MRC L 33	3.3	2.7	3	R	3	2.33	2.665	R
MRC L 34	4.5	5	4.75	MR	6.5	5.4	5.95	MS
MRC L 35	4.2	3.8	4	MR	4.5	4	4.25	MR
MRC L 36	5	4.7	4.85	MR	6	5.5	5.75	MS
MRC L 37	5	4.5	4.75	MR	5.5	5	5.25	MS
MRC L 38	7.3	6.8	7.1	S	5.8	5	5.4	MS
MRC L 39	5	4.5	4.75	MR	6	5	5.5	MS
MRC L 40	7	6.5	6.75	MS	6.2	6	6.1	MS
JCY 2-7	2.8	2.8	2.5	R	2.5	2.5	2.3	R
Kaveri-50	7.5	7.5	7.8	S	7.8	7.8	7.5	S

MRC L 25, MRC L 26, MRC L 27, MRC L 28, MRC L 30, MRC L 31, MRC L 35 has given a disease reaction of moderately resistant and the lines MRC L 2, MRC L 3, MRC L 4, MRC L 8, MRC L 10, MRC L 11, MRC L 15, MRC L 18,MRC L 21, MRC L 22, MRC L 34, MRC L 36, MRC L 37, MRC L 38, MRC L 39, MRC L 40, MRC L 16, MRC L 23, MRC L 32 has displayed the disease reaction of moderately susceptible to susceptible towards the pathogen *Fusarium verticilloides.*

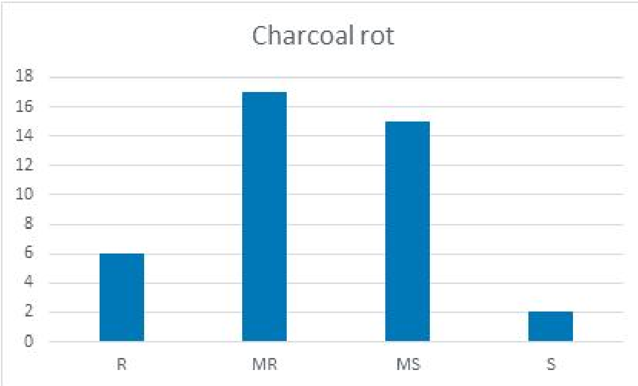

Fig. 1 : showing number of inbred lines under resistance, moderately resistance, moderately susceptible and susceptible reactions to charcoal rot disease.

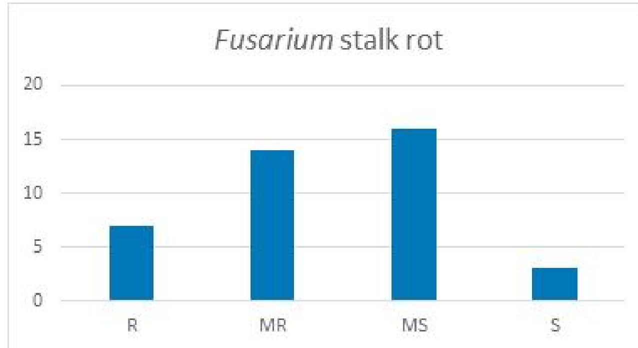

Fig. 2 : showing number of inbred lines under resistance, moderately resistance, moderately susceptible and susceptible reactions to fusarium stalk rot disease.

Plate 1 : MRC L 33 (R).

Plate 2 : MRC L 37 (MR).

Plate 3 : MRCL16 (MS).

Plate 4 : MRC L 17 (R).

Plate 5 : MRC L 1 (MR).

Plate 6 : MRC L 23(MS).

The lines MRC L 6, MRC L 13, MRC L 29, MRC L 33 has shown the resistance reaction towards both the pathogens (*Macrophomina phaseolina* and *Fusarium verticilloides*). The lines MRC L 1, MRC L 20, MRC L 24, MRC L 25, MRC L 26, MRC L 27, MRC L 30, MRC L 31 has exhibited the reaction of moderate resistance towards both the diseases and the lines MRC L 22, MRC L 32, MRC L 15, MRC L 38 has shown moderately susceptible to susceptible reaction to both the pathogens. The resistant check has shown a disease reaction of 2.5 and susceptible check has shown a reaction of 7.8. When the disease score of two seasons is compared the disease reaction is more in *kharif* than in *Rabi*. Similar results have been confirmed by Gopala *et al*. (2016) for an inbred line by following toothpick method of inoculation for both the pathogens *Macrophomina phaseolina* and *Fusarium verticilloides*. There is some difference and some similarities in the disease reaction shown by some inbreds when compared with Sravya *et al*. (2023) and Banoth *et al*. (2021) this may be due to different environmental conditions and soil conditions. Recently, Bhaskar *et al*. (2023) also found KMH-152 as the resistant maize line against *Macrophomina phaseolina* pathogen causing charcoal rot disease by following toothpick method of inoculation.

Conclusion

With the aim of identifying inbred lines that are resistant to both pathogens *Macrophomina phaseolina* and *Fusariumverticilloides* 40 maize inbred lines along with two checks were screened against two pathogens. The inbred lines MRC L 6, MRC L 13, MRC L 29, MRC L 33 were found to be resistant to both the pathogens. Many of the lines found to be moderately resistant towards *Macrophomina phaseolina* and majority of the lines were observed to be moderately susceptible to the pathogen *Fusarium verticilloides*. The resistant lines identified in the present study may be used as parents for producing hybrids as these lines were observed to be resistant to both *Macrophomina phaseolina* and *Fusarium verticilloides.*

Acknowledgement

I am very thankful to my guides for their help and guidance during this research. Their support and advice made this work possible.

References

Bhaskar, A.V. (2023). Screening for Identification of Resistant Genotypes against Charcoal Rot Caused by *Macrophomina phaseolina* in Maize (*Zea mays* L.). *Int. J. Plant Soil Sci*., **35(23)**, 222-237.

Clark, R.L. and Foley D.C. (1985). Stalk rot resistance and strength of maize stalk from the plant introduction collection. *Plant Dis*., **69**, 419–422.

Doohan, F.M., Brennan J. and Cooke B.M. (2003). Influence of climatic factors on Fusarium species pathogenic to cereals. *Eur. J. Plant Pathol*., **109**, 755-768.

Gopala, R.G., Hooda K., Rai S., Kumar A. and Hossain Firoz (2016). Rapid screening technique for evaluation of maize genotypes against stalk rot complex caused by *Macrophomina phaseolina* and *Fusarium verticilloides*. *Indian J. Agric. Sci*., **86(8)**, 1024-1030.

Hooda, K.S., Bagaria P.K., Khokhar M., Kaur H. and Rakshit S. (2018). *Mass screening techniques for resistance to maize diseases*. ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana-141004, 93.

Hooda, K.S., Sekhar J.C., Chikkappa G., Kumar Sangit, Pandurange K.T., Sreeramsetty T.A., Sharma S.S., Kaur H., Gogoi R., Reddy R. and Kumar P. (2012). Identifying sources of multiple disease resistance in maize. *Maize J*., **1(1)**, 82-84.

https://agriexchange.apeda.gov.in/

https://www.fao.org/policy-support/tools-and-publications/resources-details/en/c/1330453/

https://www.indiastat.com/maize/production/area.

Khokhar, M.K., Hooda K.S., Sharma S.S. and Singh V. (2014). Post Flowering Stalk Rot Complex of Maize - Present Status and Future Prospects. *Maydica*, **59**, 226-242.

Krishna, K.M., Chikkappa G.K. and Manjulatha G. (2013). Components of genetic variation for *Macrophomina phaseolona* resistance in maize. *J. Res. ANGRAU*, **41(3)**, 12-15.

Laxmi Sravya, T., Seshu G., Yella Goud T. and Nagesh Kumar M.V. (2023). Screening of some maize cultivars for charcoal rot in Northern Region of Telangana, India. *Int. J. Plant Soil Sci.*, **35(19)**, 1542-1548.

Madhu, Banoth, Prabhavathi K., Bhadru D. and Mallaiah B. (2021). Screening Technique for Identification of Resistant Genotypes against Post Flowering Stalk Rot Complex caused by *Macrophomina phaseolina* in Maize (*Zea mays* L.). *Int. J. Curr. Microbiol. Appl. Sci*., **10(01)**, 3535-3544.

Nyvall, R.F. and Kommedahl T. (1970). Saprophytes and survival of *Fusarium moniliforme* in corn stalks. *Phytopath.*, **60**, 1233-1235

Pascale, M., Visconti A. and Chelkowsky J. (2002). Ear rot susceptibility and mycotoxin contamination of maize hybrids inoculated with *Fusarium* species under field conditions. *Eur. J. Plant Pathol*., **108**, 645-651.

Payak, M.M. and Sharma R.C. (1985). Maize diseases and approaches to their management in India. Int. *J. Pest Manage*., **31**, 302-310.

Payak, M.M. and Sharma R.C. (1983). Disease rating scales in maize in India. In : *Techniques of Scoring for Resistance to Diseases of Maize in India*. All India Co-ordinated Maize Improvement Project, IARI, NewDelhi. 1–4.

Raju, C.A. and Lal S. (1976). Relationship of *Cephalosporium acremonium* and *Fusarium moniliforme* with stalk rots of maize. *Indian Phytopathol*., **29(3)**, 227-231

Sandra, G.A., Carolina V.F., Katy D.P., José H.C., Matilde U.M. and Eugenio S.V. (2008). Identification and biological characterization of isolates with activity inhibitive against *Macrophomina phaseolina* (Tassi) goid. *Chil. J. Agric. Res*., **69(4)**, 526-533.

Shekhar, M., Kumar S., Sharma R.C. and Singh R. (2010). Sources of resistance against post-flowering stalk rot of maize. *Arch. Phytopathol and Plant Pflanzenschutz* **43**, 259–263.