Int.J.Curr.Microbiol.App.Sci (2018) *7*(2): 3375-3383

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 02 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.702.404

Disease Reaction Studies of Heat Tolerant Maize (*Zea mays* L.) Inbred Lines under Artificial Epiphytotic Conditions against Turcicum Leaf Blight (*Excerohilum turcicum*) and *Aspergillus flavus* Contamination

K. Ajithkumar[1*], A.S. Savitha[2], Prakash H. Kuchanur[3] and B. Rajanna[1]

[1]Main Agricultural Research Station, [2]Department of Plant Pathology, [3]Department of Genetics and Plant Breeding, University of Agricultural Sciences, Raichur, Karnataka–584 104, India

**Corresponding author*

Keywords

Aspergillus, Inbred lines, Maize, Evaluation, Turcicum leaf blight

Article Info

Accepted:
26 January 2018
Available Online:
10 February 2018

A B S T R A C T

Among biotic stresses affecting maize, the pre-harvest aflatoxin contamination produced by *Aspergillus flavus* and turcicum leaf blight (TLB) caused by *Excerohilum turcicum* are most important diseases in India. Resistance of maize to aflatoxin contamination and TLB, is a penultimate goal in breeding programs that screen for these important traits with the aim of developing resistant commercial hybrids. Hence a study on reaction of 26 inbred lines of heat tolerant maize to both the diseases was conducted under artificial epiphytotic field conditions for two seasons. Among 26 inbred lines evaluated for TLB, 10 lines namely CAH-1533, CAH-1505, CAH-158, CAH-1437, H-15002, E-5, CAH-1526, CAH-1454, CAH-1532 and CAH-1545 showed resistant reaction. Whereas five lines were found moderately resistant *viz*., CAH-1551, CAH-1504, CAH-1564, CAH-1536 and CAH-1501 and remaining were severely affected by TLB and rated them as susceptible. Study on reaction of 28 inbred lines for aflatoxin contamination revealed that, among them none were immune, whereas nine lines namely CAH-1546, CAH-1525, H-15001, ARLUM, CAH-1437, CAH-1503, CAH-1526, CAH-1545 and CI-4 were highly resistant to aflatoxin contamination. The resistant reaction was exhibited by 17 lines, while two inbred lines namely CAH-1551 and CAH-1501 showed moderately resistant to aflatoxin contamination. Thus the above lines were identified as promising sources of resistance and can be used successfully in developing desirable level of resistance in disease endemic areas to aim for sustainable productivity.

Introduction

Maize (*Zea mays* L.) is an important staple food crop and provides raw materials for the livestock and many agro- allied industries in the world (Bello *et al.*, 2010; Randjelovic *et al.*, 2011). Globally, maize is known as "Queen of Cereals" because it has the highest genetic yield potential among the cereals. It is commonly known as corn in the United States and Canada and is the third most important cereal grain worldwide after wheat and rice (Golob *et al.*, 2004). It is referred to as the cereal of the future for its nutritional value and utility of its products and by-products (Lee, 1999). The area, production and productivity

of maize has increased significantly in the last few decades. India registered a growth rate of more than seven per cent in production and more than six per cent in productivity in the last five years.

In India, maize is the third most important food crops after rice and wheat. According to advance estimate it is cultivated in an area of 9.23 m ha by producing 23.67 m t (2015-16) mainly during *Kharif* season which covers 80 per cent area in the states like Karnataka, Andhra Pradesh, Uttar Pradesh, Bihar, Rajasthan, Madhya Pradesh and Punjab.

The average maize yield in India is 2.56 t ha^{-1}, which is much lower than most of the maize growing countries of the world (www.agricoop.nic.in). Karnataka state accounts for 1.38 m ha area with a production of 3.98 million tons and 2883 kg ha^{-1} productivity (Anon., 2014). Karnataka has six per cent of the total maize area with 12 per cent share in production in the country.

For many years, maize is used as food for human and different animals. Therefore, maize breeders bestow great and uninterrupted efforts to improve and increase the yielding ability. Several pathogens are known to cause diseases in maize plants. The diseases of maize caused by fungi are of great economic importance, of which TLB of maize caused by *Excerohilum turcicum* is an important foliar disease. The disease (TLB) is endemic in all maize growing areas and considered as a foremost limiting biotic factor for successful cultivation of maize, which results in significant yield losses in the range of 28 to 91 per cent (Pant *et al.,* 2000; Singh *et al.,* 2004). The fungus *Aspergillus flavus* is responsible for both pre- and post-harvest accumulation of aflatoxin in maize. Of all the known mycotoxins – toxic substances produced by fungi, aflatoxins in maize and other food items such as milk, peanut, or cotton meal *etc*., generate the most concern when they occur at high levels. Resistance against *A. flavus* infection and aflatoxin accumulation is a complex trait that is influenced by genotype, agronomic practices and environmental conditions. Beneficial secondary traits such as husk covering and tightness, physical properties of the pericarp and drought or heat stress tolerance are factors contributing to aflatoxin resistance. Among the various management mechanisms studied, host-plant resistance is supposed to be the best and widely explored strategy. It focuses on inhibition of fungal colonization and/or toxin production by fungus on the host plant, by the development of resistant inbreds (Brown *et al.,* 2003). It would also eliminate the need to detoxify large quantities of aflatoxin-contaminated seeds. The utilization of such resistant varieties has been the hope for developing resistant genotypes.

Materials and Methods

A field experiment was conducted to screen the 26 heat tolerant maize inbred lines which were developed as the part of adhoc project entitled "Testing and Deployment of New Drought Resilient Maize Hybrids for Rainfed Regions of Karnataka, India" at Dept of Genetics and Plant Breeding, College of Agriculture, Bheemarayanagudi to identify the resistant sources for aflatoxin contamination and TLB breeding programmes. The inbred lines were sown with 60 cm spacing between line and 30 cm spacing between plants and to facilitate the good inoculums of *E. turcicum* to build up one resistant check (CI-4) and one susceptible check (CM-202) were sown for every 10 inbred lines at Main Agricultural Research Station experimental block, University of Agricultural Sciences, Raichur during two seasons of *Kharif* - 2015 and *Kharif* - 2016. The recommended agronomic practices as well required plant protection measures were followed to raise a good crop.

Screening for TLB was carried out by artificially inoculating plants at 5-6 leaf stage with the conidial suspension of test fungus (*E. turcicum*) 4-5 x 104 conidia ml^{-1} in the evening hours and high humidity was maintained by spraying water 5-6 times in the next 3 days to ensure infection.

The intensity of disease in the field was estimated from five randomly selected plants in each genotype which were tagged with labels at the flowering stage of the crop. On an average 10 leaves were selected at random from the selected plant and disease severity was recorded by visually examining each leaf and the disease severity was scored by using 0-5 scales adopted by maize pathology unit, CIMMYT as follows in Table 1.

Further, the per cent disease severity was calculated by using the formula given by Wheeler (1969).

Per cent disease severity = (Sum of all observation grades / Total number of plants observed) × (100 / Maximum grade).

Screening of maize inbreed lines against aflatoxin contamination will be done by Silk Channel Inoculation Assay (SCIA) – syringe technique developed by Verderio *et al.,* (2007) on adult plants. *A. flavus* was grown on PDA plates at 26 ℃ until the mycelium covered the surface of the plate and used for fresh spore inoculum production. For the field experiments, plants were hand-pollinate and SCIA was applied at two different stages of kernel development at 3 and 6 days after pollination. For the SCIA tests, concentration of conidia was determined with a hemacytometer and adjusted to 9 x 10^7 conidia ml^{-1} with sterile distilled water. Inoculation was performed by spore injections into the silk channel of each primary ear. Ears were manually harvested, hand de-husked; the severity of ear rot symptoms evaluated using rating scales developed by Reid *et al.,* (1996) (Table 2) based on the percentage of kernels with visible symptoms of infection, such as rot and mycelium growth.

The per cent disease index was calculated by using the formula given by Wheeler (1969),

Per cent disease index = (Sum of all observation grades / Total number of plants observed) × (100 / Maximum grade).

Results and Discussion

Continuous efforts to locate resistant source and utilization in resistant breeding programme are imperative to manage the disease in the long run. The screening trial revealed that, none of the tested inbred lines were completely free from TLB infection. However, significant variations in disease severity index for TLB was observed in inbred lines. The experiment was conducted during *Kharif* - 2015 and *Kharif* – 2016 revealed that out of 26 inbred lines tested, none were free from the TLB disease, whereas 10 lines *viz*., CAH-1533, CAH-1505, CAH-158, CAH-1437, H-15002, E-5, CAH-1526, CAH-1454, CAH-1532 and CAH-1545 have disease score of 1 with the per cent disease severity ranging from 4.33 to 8.67 thereby exhibited resistant reaction. However five lines *viz*., CAH-1551, CAH-1504, CAH-1564, CAH-1536 and CAH-1501 were showed the per cent disease severity of 16.33 to 24.33 which have disease score of 2 with moderately resistant to the disease and remaining were severely affected by TLB having disease score of more than 3 with per cent disease severity of above 26 and categorized under susceptible group (Table 3).

An experiment was conducted during *Kharif* - 2015 and *Kharif* – 2016 to know the resistant expression of 28 maize inbred lines for aflatoxin contamination reveals that, not even a single inbred line was recorded immune reaction.

Table.1 Disease severity rating for screening of maize lines against TLB infection

Disease Severity Rating	Per cent of leaf area covered with lesions	Reaction
0	No symptoms	Highly Resistant (HR)
1	One to few scattered lesions on leaves covering up to 10% leaf area	Resistant (R)
2	Lesions on leaves covering 11-25% leaf area	Moderately Resistant (MR)
3	Lesions on leaves covering 26-50% leaf area	Moderately Susceptible (MS)
4	Lesions abundant on leaves covering 51-75% leaf area	Susceptible (S)
5	Lesions abundant on almost all leaves, plants prematurely dried or killed with 76-100% leaf area covered	Highly Susceptible (HS)

Table.2 Visual rating scale for screening maize lines against aflatoxin

Disease Severity Rating	Per cent of infected kernels	Reaction
1	0	Immune
2	1-3	Highly Resistant (HR)
3	4-10	Resistant (R)
4	11-25	Moderately Resistant (MR)
5	26-50	Moderately Susceptible (MS)
6	51-75	Susceptible (S)
7	76-100	Highly Susceptible (HS)

Table.3 Reaction of maize inbred lines for turcicum leaf blight infection

Sl. No.	Inbred lines	TLB infection		
		Severity (%)	Disease Rating	Reaction
1	CAH-1551	16.33	2	MR
2	CAH- 1533	8.67	1	R
3	CAH-1546	32.33	3	MS
4	CAH-1505	4.33	1	R
5	CAH-1525	36.67	3	MS
6	H-15001	44.00	3	MS
7	CAH-1579	48.33	3	MS
8	H-15003	84.33	5	HS
9	ARLUM	92.67	5	HS
10	CAH-1535	28.67	3	MS
11	CAH-158	4.67	1	R
12	CAH-1506	64.33	4	S
13	CAH-1437	8.33	1	R
14	H-15002	8.67	1	R
15	E-5	4.67	1	R
16	CAH-1503	68.00	4	S
17	CAH-1541	32.33	3	MS
18	CAH-1526	6.33	1	R
19	CAH-1454	8.67	1	R
20	CAH-1504	24.33	2	MR
21	CAH-1564	16.67	2	MR
22	CAH-1545	8.33	1	R
23	CAH-1536	20.67	2	MR
24	CAH-1502	68.67	4	S
25	CAH-1501	20.33	2	MR
26	CAH-1532	8.67	1	R
27	CM-202 (SC)	73.45	4	S
28	CI-4 (RC)	4.00	1	R

Table.4 Reaction of maize inbred lines for *Aspergillus* contamination

Sl. No.	Inbred lines	*Aspergillus* contamination		
		% seed infected	Disease Rating	Reaction
1	CAH-1551	10.21	4	MR
2	CAH- 1533	4.91	3	R
3	CAH-1546	1.01	2	HR
4	CAH-1505	4.36	3	R
5	CAH-1525	1.88	2	HR
6	H-15001	5.42	3	R
7	CAH-1579	3.73	3	R
8	H-15003	1.65	2	HR
9	ARLUM	2.33	2	HR
10	CAH-1535	3.20	3	R
11	CAH-158	3.76	3	R
12	CAH-1506	3.27	3	R
13	CAH-1437	1.25	2	HR
14	H-15002	3.24	3	R
15	E-5	6.20	3	R
16	CAH-1503	2.34	2	HR
17	CAH-1541	5.90	3	R
18	CAH-1526	2.85	2	HR
19	CAH-1454	3.76	3	R
20	CAH-1504	6.89	3	R
21	CAH-1564	3.15	3	R
22	CAH-1545	2.33	2	HR
23	CAH-1536	5.06	3	R
24	CAH-1502	7.57	3	R
25	CAH-1501	22.18	4	MR
26	CAH-1532	3.81	3	R
27	CM-202	8.13	3	R
28	CI-4	2.42	2	HR

Table.5 Grouping of maize inbred lines based on their degree of resistance

Sl. No.	Degree of Resistance	TLB Infection	*Aspergillus* contamination
1	Highly Resistant	--	CAH-1546, CAH-1525, H-15003, ARLUM, CAH-1437, CAH-1503, CAH-1526, CAH-1545 and CI-4 (09).
2	Resistant	CAH-1533, CAH-1505, CAH-158, CAH-1437, H-15002, E-5, CAH-1526, CAH-1454, CAH-1532 and CAH-1545 (10).	CAH-1533, CAH-1505, H-15001, CAH-1579, CAH-1535, CAH-158, CAH-1506, H-15002, E-5, CAH-1541, CAH-1454, CAH-1504, CAH-1564, CAH-1536, CAH-1502, CAH-1532 and CM-202 (17).
3	Moderately Resistant	CAH-1551, CAH-1504, CAH-1564, CAH-1536 and CAH-1501 (05).	CAH-1551 and CAH-1501 (02).
4	Moderately Susceptible	CAH-1546, CAH-1525, H-15001, CAH-1579, CAH-1535 and CAH-1541 (06).	--
5	Susceptible	CAH-1506, CAH-1503 and CAH-1502 (03)	--
6	Highly Susceptible	H-15003 and ARLUM (02).	--

Among 28 lines, nine inbred lines namely CAH-1546, CAH-1525, H-15003, ARLUM, CAH-1437, CAH-1503, CAH-1526 CAH-1545 and CI-4 have disease score of 2 with the per cent seed infection ranging from 1.01 to 2.85 thereby exhibited highly resistant reaction, whereas resistant reaction was exhibited by 17 lines by recording disease score of 3 with per cent seed infection ranging from 3.15 to 8.13 while two inbred lines namely CAH-1551 and CAH-1501 found moderately resistant having disease score of 4 with per cent seed infection of aflatoxin contamination ranging from 10.21 to 22.18 (Table 4). As anticipated, some lines showed resistance only to aflatoxin rather than TLB infection (Table 5). These lines may show different degrees of resistance when examined in other localities or may be useful for breeding for resistance to individual diseases. These lines may also be useful for mapping populations focused on identifying quantitative trait loci (QTL) involved in resistance to individual fungi. Of particular interest among the lines examined in the study were CAH-1533, CAH-1505, CAH-158, CAH-1437, H-15002, E-5, CAH-1526, CAH-1454, CAH-1545 and CAH-1532 which exhibited resistant reactions to both aflatoxin contamination and TLB infection. These 10 lines with consistent resistance to aflatoxin and TLB infection may, therefore, be useful sources of resistance for maize breeding programs to reduce both aflatoxin contamination and TLB infection.

Disease reaction indicating satisfactory level of disease development and the categorization of materials into different classes was appropriate (Table 5). The results are in accordance with Ramdutta and Sangam Lal (2005) who screened maize inbred lines under glasshouse condition and observed that the lines were resistant to *E. turcicum.* These

findings were in agreement with results obtained by Chandrashekara *et al.*, (2012) and Soumya Goudar and Harlapur (2016) while working with *Turcicum* leaf blight of maize.

These results support a previous report that *A. flavus* can concurrently contaminate corn ears with aflatoxin. It had previously been suggested by Ubwa *et al.*, (2012) that there was no significant positive or negative correlation between *A. flavus* and *E. turcicum* in maize (Sangit Kumar *et al.*, 2011).

Thus, the promising high yielding resistant genotypes identified through this investigation would be helpful for their deployment in breeding program and as donors for different basic and applied research programmes and could be used to develop lines for *A. flavus* and *E. turcicum* disease endemic areas to aim at sustainable productivity.

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How to cite this article:

Ajithkumar, K., A.S. Savitha, Prakash H. Kuchanur and Rajanna, B. 2018. Disease Reaction Studies of Heat Tolerant Maize (*Zea mays* L.) Inbred Lines under Artificial Epiphytotic Conditions against Turcicum Leaf Blight (*Excerohilum turcicum*) and *Aspergillus flavus* Contamination. *Int.J.Curr.Microbiol.App.Sci.* 7(02): 3375-3383.
doi: https://doi.org/10.20546/ijcmas.2018.702.404