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Evaluation of maize hybrids and inbred lines for resistance to pre-harvest aflatoxin and fumonisin producing fungal contamination in the field

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Abstract

Resistance of maize to pre-harvest mycotoxin contaminating fungi, *Aspergillus flavus* and *Fusarium verticillioides*, is a goal in breeding programs that screen for these important traits with the aim of developing resistant commercial hybrids. We conducted two years of field experiment for evaluation of 125 advanced breeding lines of maize. Aflatoxin and fumonisin producing fungi were present in majority of the samples harvested from all lines in both the years. The results of the maize germplasm screening revealed that, out of 125 germplasm screened none of them showed immune and highly resistant response to mycotoxigenic fungi, whereas only one germplasm from the pool of CIMMYT hybrids namely Z979-38 showed resistant reaction only against *A. flavus* but moderately resistant to *F. verticillioides.* These selected lines, particularly Z979-38, may provide sources of resistance to *A. flavus* contamination in breeding programs. However, the mechanism of resistance in this germplasm remains to be identified.

Keywords: Aflatoxin, fumonisin, mycotoxin, maize, hybrids, germplasm

Introduction

Maize is one of the staple food crops after wheat and rice. It is referred to as the cereal of the future for its nutritional value and utility of its products and by-products (Lee, 1999) [12]. 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.

According to advance estimate maize area of 9.86 m ha with a production of 26.26 million tons having a productivity of 2664 kg/ha (Anon., 2018) [5]. The predominant maize growing states that contribute more than 80 per cent of the total maize production are Karnataka (17.2 %), Andhra Pradesh (14.9 %), Rajasthan (9.9 %), Maharashtra (9.1 %), Bihar (8.9 %), Uttar Pradesh (6.1 %), Madhya Pradesh (5.7 %) and Himachal Pradesh (4.4 %) (www.thedailyrecords.com, 2019). Apart from these states maize is also grown in Jammu and Kashmir and North-Eastern states. However, in West Bengal, maize productivity was 4059 kg / ha with a production of 522.4 thousand tons from total area of 128.7 thousand ha (Anon., 2014) [6].

A. flavus (Link ex Fr.) and *F. verticilliodes* (Sacc.) Nirenberg (syn.: *F. moniliforme*) are the two predominant ear-rotting pathogens of maize (*Zea mays* L.) in India. Damage caused by ear-feeding pests can provide an entrance for fungal infection leading to subsequent mycotoxin contamination (such as by aflatoxin and fumonisin) of grain. Mycotoxin contamination results in severe yield losses, reduces crop quality and poses a significant threat to human and animal food safety. Aflatoxins are powerful hepatotoxins, teratogens, mutagens and carcinogens on the other hand fumonisins have been reported to induce several diseases in animals, notably leukoencephalomalacia in horses, pulmonary edema in swine and liver cancer in human (Abbas *et al*., 2002) [2]. The U.S. Food and Drug Administration (FDA) regulates aflatoxin B1, the most common form of aflatoxin found in maize, at less than 20 µg/kg for human consumption, whereas total fumonisin levels in human food and animal feed are regulated at less than 2 mg/kg (Anon., 2016) [7]. Although both mycotoxins can be found in the same maize ear, the relationship between aflatoxin and fumonisin contamination is not well understood. Marin *et al.* (1998) [13] found that the growth of *Aspergillus* spp. was slowed in the presence of

Fusarium spp., showing a competitive relationship and a negative correlation between the growth of *A. flavus* and *F. verticillioides*.

In contrast, Abbas *et al.* (2006) [1] found that aflatoxin and fumonisin levels were positively correlated across test environments in hybrids naturally infected with both *Fusarium* spp. and *A. flavus*. These results suggest that both fungi can thrive on similar resources if host plants are highly susceptible.

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) [8]. 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.

Breeding techniques employed for maize have developed and advanced over time and include conventional breeding, mutation breeding and molecular-assisted breeding (Including transgenics and molecular markers). Conventional breeding has made a significant contribution to increased maize production through the development of hybrids with resistance to abiotic stresses such as drought, heat and cold with partial resistance to aflatoxin and fumonisin.

The evaluation and identification of maize lines with reduced aflatoxin and fumonisin contamination will assist in breeding for improvement of maize resistance to mycotoxin contamination. Efforts to develop inbred lines resistant to mycotoxin contamination in grains have been ongoing since the mid-1970s.

Materials and Methods

Silk inoculation technique

Screening of maize breeding lines for identification of resistance sources will be done by Silk Channel Inoculation Assay (SCIA) – syringe technique developed by Verderio *et al.* (2007) [16] on adult plants. The mycotoxin producing fungus (*A. flavus* and *F. verticillioides*) were grown on PDA plates at 26 °C until the mycelium covered the surface of the plate and used for fresh spore inoculum production. For the field experiments, plants were hand-pollinated and SCIA will be applied at two different stages of kernel development at 3 and 6 days after pollination (DAP); controls were uninoculated and sterile water-inoculated plants. For the SCIA tests, concentration of conidia will be determined with a hemacytometer and adjusted to 9×10^7 conidia per ml with sterile distilled water. Inoculum not used immediately was stored at 4 °C. Inoculation was performed by spore injections into the silk channel of each primary ear.

Inoculation of cobs

For screening purpose 125 advanced breeding lines including 13 UASR hybrids, 45 CIMMYT hybrids and 67 inbreds of CIMMYT were used. Before sowing, seeds of all the germplasm were treated with captan. The germplasm were sown in two replication, about five cobs were selected for each *A. flavus* and *F. verticillioides* from each replication and were inoculated with the spore suspension of respective fungi and were labelled.

Cobs were manually harvested, hand de-husked and the severity of the mycotoxin producing fungus was evaluated using rating scales based on the percentage of kernels with visible symptoms of infection, such as rot and mycelium growth. As reported by Reid *et al.* (1996) [14], the visual rating scale consists of seven classes based on percentage of visibly infected kernels was employed for grouping of germplasm (Table 1) by using the formula given below.

$$\text{Per cent infection of grain per cob} = \frac{\text{Number of infected grains}}{\text{Total number of grains observed}} \times 100$$

Table 1: Visual disease scale for rating occurrence of mycotoxin contamination

Disease Severity Rating	Per cent infected grains	Reaction
1	0	Immune
2	1-3	Highly Resistant
3	4-10	Resistant
4	11-25	Moderately resistant
5	26-50	Moderately susceptible
6	51-75	Susceptible
7	76-100	Highly susceptible

Results and Discussion

In 2017-18 and 2018-19, 125 maize germplasm which included 13 UASR hybrids, 45 CIMMYT hybrids and 67 inbreds of CIMMYT were screened in the field for aflatoxin and fumonisin producing fungal contamination, with five replicates each year. Samples from all the examined lines were contaminated with aflatoxin and fumonisin producing fungi.

Screening of maize germplasm for *A. flavus* infection during 2017-18

Large variations were observed in seed colonization severity (3.32-34.63 %) among the germplasm belonging to different sections and species (Table 2). Out of 125 germplasm, none were immune against mycotoxin producing *A. flavus*, while two CIMMYT hybrids namely Z979-31 and Z979-7 and one inbred line, CML-286 showed highly resistant reaction. Eleven germaplsm including 10 CIMMYT hybrids such as Z979-22, Z979-29, Z979-2, LC-1, Z979-48, Z979-23, Z979-37, Z979-38, Z979-13 and Z979-24 and one inbred line (E1 CIMMYT B'gudi) exhibited resistant response. Among the remaining, 102 germplasm recorded moderately resistant reaction, but nine germplasm belonged to moderately susceptible group. The germplasm pool consisting of 13 UASR hybrids, eventually showed the moderately resistant reaction.

Screening of maize germplasm for *F. verticilloides* infection during 2017-18

The reaction of 125 germplasm against *F. verticilloides* infection has wide variation from 4.88 to 34.47 per cent disease severity (Table 3). Out of 125 germplasm, immune and highly resistant response was not indicated by any of the germplasm, whereas 10 germplasm including eight germplasm from CIMMYT hybrids such as Z979-53, Z979-50, Z979-51, Z979-29, Z979-23, Z979-37, Z979-20 and Z979-17 and two inbred lines namely CML-286 and E1 CIMMYT B'gudi showed resistant reaction. Among the rest of the 115 germplasm, 107 germplasm were categorised with moderately resistant reaction, while eight germplasm fit in to moderately susceptible group. The reaction of 13 UASR hybrids against *F. verticilloides* infection was also witnessed for the moderately resistant reaction.

Screening of maize germplasm for *A. flavus* infection during 2018-19

Similar sets of 125 germplasm of maize screened for *A. flavus* infection to confirm their reaction during 2018-19. None of the germplasm showed immune reaction and highly resistant response, whereas only one germplasm from the pool of CIMMYT hybrids namely Z979-38 showed resistant reaction. In the remaining germplasm collection of 124, the moderately resistant response was exhibited by 103 germplasm with 21 germplasm expressing moderately susceptible reaction (Table 2).

Screening of maize germplasm for *F. verticilloides* infection during 2018-19

The response of the 125 maize accessions against *F. verticilloides* infection is presented here under. No resistant source was identified in the accessions pool, where none of the germplasm showed immune, highly resistant and resistant response. Out of 125 germplasm screened, nearly half of the population (63 germplasm) showed moderately resistant reaction whereas 62 germplasm showed moderately susceptible reaction (Table 3).

The two years pooled results of the maize germplasm screening revealed that, out of 125 germplasm screened for *A. flavus* only one germplasm from the group of CIMMYT hybrids namely Z979-38 showed evidenced for resistant reaction. Among the remaining germplasm, 99 were assembled under moderately resistant response whereas 25 germplasm showed moderately susceptible reaction. The similar trend was also recorded in the two years pooled results of the germplasm when they were screened against *F. verticilloides* infection, where none of the germplasm fall in the group of immune, highly resistant and resistant reaction. Out of the 125 germplasm, 59 germplasm were accumulated under moderately resistant response whereas 66 germplasm showed moderately susceptible reaction (Table 4 and 5).

Alleviation of mycotoxin contamination through genetic manipulation has been attempted in most of the maize producing countries since late 1960s. Breeding resistant cultivar is possible only when there are high-level of resistance sources stable in nature. It is very important that screening methods provide reliable information on the responses of various genotypes. Fungicides though necessary to manage many diseases but is undesirable and often uneconomical as a long term solution to the health of living beings. Therefore, efforts in identification of resistant genotypes would make other measures more effective. Identification of resistance sources is amplified in biotrophs and hemibiotrophs pathogen but it is not successful in case of nectrotrphs to which mycotoxigenic fungi belongs. The interaction between pathogen and host is very less due to restricted immune system at the seed level.

The results of the study is supported by previous report (Robertson *et al*., 2007) [15] that *A. flavus* and *F. verticillioides* can concurrently contaminate corn ears with aflatoxin and fumonisin, respectively. It had previously been suggested that there was no significant positive or negative correlation between *Aspergillus* spp. and *Fusarium* spp. in maize. The natural infection of maize with *Fusarium* spp. did not appear to influence the production of aflatoxin by *A. flavus* with the inoculation method used. These differences in observations may result from either lower levels of *Fusarium* spp. infection or high levels of *A. flavus* infection associated with artificial inoculation.

Dhiraj *et al*. (2017) [9] transformed maize plants with a kernel-specific RNA interference (RNAi) gene cassette targeting the aflC gene, which encodes an enzyme in the *Aspergillus* aflatoxin biosynthetic pathway. After pathogen infection, aflatoxin could not be detected in kernels from these RNAi transgenic maize plants, while toxin loads reached thousands of parts per billion in non-transgenic control kernels.

However, contrary to the work of Ajithkumar *et al.* (2018) [4] who screened heat tolerant maize inbred by employing silk inoculation technique against *A. flavus*. Among 28 inbred lines 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. The resistant reaction was exhibited by 17 lines, while two inbred lines namely CAH-1551 and CAH-1501 showed moderately resistant to *A. flavus*.

Identification of the traits that contribute for resistance against *A. flavus* and *F. verticillioides* is an important task in breeding programs. Hence, it is critical to evaluate the collection of germplasm sources for resistance in order to advance the development of commercial hybrids with resistance to mycotoxin contamination across years. The result was supported when concentrations of aflatoxin and fumonisin were compared in 87 inbred lines by Guo *et al.* (2017) [11], identified several inbred lines that have served as sources of resistance and other agronomic traits in breeding programs. Mp717 was developed and released as a source of resistance to *A. flavus* infection and aflatoxin accumulation and served as the resistant control in this study (Williams and Windham, 2009) [17]. Among the lines examined, 53 showed aflatoxin levels equivalent or lower than those of Mp717 suggesting that these lines may be useful in breeding efforts to enhance aflatoxin resistance.

As anticipated, some germplasm showed resistance only to aflatoxin rather than to fumonisin contamination and *vice versa*. These lines may show different degrees of resistance when examined in other environments or may be useful for breeding for resistance to individual mycotoxins. Such lines may also be useful for mapping populations focused on identifying quantitative trait loci (QTL) involved in resistance to individual mycotoxigenic fungi. Of particular interest among the lines examined in the study were CN1, GT601 (Guo *et al*., 2011) [10] TUN09 and TUN61, which exhibited total fumonisin levels less than 2 mg/kg and total aflatoxin concentrations lower than that of Mp717. These four lines with consistent resistance to aflatoxin and fumonisin may, therefore, be useful sources of resistance for maize breeding programs to reduce both contamination of aflatoxin and fumonisin.

Although these experiments indicated a positive overall relationship between aflatoxin and fumonisin contaminations, many questions remain to be unaddressed in order to elucidate the relationship between both mycotoxins and their producing organisms and one possibility is to use RT-PCR to quantify the fungal biomass of each fungal species and fungal infection rate. The possibility of common host resistance mechanisms of maize, as well as common infection and virulence mechanisms of the fungi, should be investigated.

Other factors that may influence these host-pathogen interactions, such as abiotic stress and insect feeding damage, should be considered. Abbas *et al*. (2004) [3] observed that both *Aspergillus* and *Fusarium* ear rot were significantly associated with insect damage. Genes that control plant stress reactions may also contribute to the correlation between aflatoxin and fumonisin levels.

Conclusions

The contamination of agricultural crops with aflatoxin and fumonisin is a major concern for global food security. Breeding for resistance is still considered to be one of the best strategies currently available to lower aflatoxin and fumonisin accumulation in maize. In the present study, we identified 99 germplasm against *A. flavus* and 59 germplasm against *F. verticillioides* that showed moderate resistant reaction but Z979-38 was resistant to *A. flavus*. An interesting note is that resistance to the mycotoxigenic fungi contamination has now been documented and this resistance would be a significant step forward for potentially breeding hybrids with resistance to aflatoxin and fumonisin accumulation. Identifying maize germplasm resistant to both aflatoxin and fumonisin contamination, would make it possible to enhance the resistance of maize hybrids in breeding programs and would allow further study of the specific mechanisms underlying resistance to both *A. flavus* and *F. verticillioides.*

Table 2: Screening of breeding lines of maize against *A. flavus*, mycotoxin producing fungi

Sl. No.	Maize Germplasm	Seed Infection (%)		Pooled Seed infection (%)	Reaction
		2017	2018		
UASR Hybrids					
1	CAH-1437	16.29	23.17	23.17	MR
2	CAH-1525	15.21	17.56	17.56	MR
3	CAH-1526	13.90	16.28	16.28	MR
4	CAH-1545	15.27	17.46	17.46	MR
5	CP-818	13.56	20.30	20.30	MR
6	900M	14.58	15.75	15.75	MR
7	E-1	12.53	14.00	14.00	MR
8	E-2	16.12	17.99	17.99	MR
9	E-3	15.38	17.16	17.16	MR
10	NK-6240	16.20	17.95	17.95	MR
11	Z-788-5	17.30	18.81	18.81	MR
12	Z-788-19	19.73	20.48	20.48	MR
13	Z-813-8	11.89	12.90	12.90	MR
CIMMYT Hybrids					
1	Z979-31	3.91	22.36	22.36	MR
2	Z979-53	15.33	16.98	16.98	MR
3	Z979-45	14.56	16.29	16.29	MR
4	Z979-22	4.82	12.33	12.33	MR
5	Z979-50	17.47	19.21	19.21	MR
6	Z979-29	6.45	15.95	15.95	MR
7	Z979-6	12.63	13.62	13.62	MR
8	Z979-51	14.05	15.28	15.28	MR
9	Z979-49	19.37	14.67	19.37	MR
10	Z979-7	3.32	15.61	15.61	MR
11	Z979-46	20.57	20.09	20.57	MR
12	Z979-28	17.02	18.46	18.46	MR
13	LC-3	18.27	20.58	20.58	MR
14	Z979-8	17.86	21.16	21.16	MR
15	Z979-2	6.04	13.49	13.49	MR
16	Z979-30	28.17	32.28	32.28	MS
17	LC-1	5.87	12.85	12.85	MR
18	Z979-40	20.08	21.53	21.53	MR
19	Z979-39	23.99	26.98	26.98	MS
20	Z979-16	21.44	24.51	24.51	MR
21	Z979-48	11.78	12.81	12.81	MR
22	Z979-23	5.30	12.42	12.42	MR
23	Z979-38	8.24	9.84	9.84	R
24	Z979-1	16.58	18.38	18.38	MR
25	Z979-37	5.35	14.74	14.74	MR
26	Z979-36	16.22	18.30	18.30	MR
27	Z979-20	16.76	19.72	19.72	MR
28	Z979-27	16.10	17.97	17.97	MR
29	Z979-17	13.47	15.38	15.38	MR
30	Z979-15	19.62	22.93	22.93	MR
31	Z979-04	24.76	28.18	28.18	MS
32	Z979-34	19.56	20.83	20.83	MR
33	Z979-19	30.37	22.59	30.37	MS
34	Z979-10	12.57	14.76	14.76	MR
35	Z979-21	22.07	22.05	22.07	MR
36	Z979-25	21.34	26.09	26.09	MS
37	LC-2	12.15	13.62	13.62	MR
38	Z979-13	5.87	20.63	20.63	MR
39	Z979-41	16.13	18.28	18.28	MR
40	Z979-47	18.60	20.74	20.74	MR

41	Z979-24	6.51	16.79	16.79	MR
42	Z979-9	18.87	19.83	19.83	MR
43	Z979-35	13.45	14.92	14.92	MR
44	Z979-1	15.71	17.65	17.65	MR
45	Z979-54	21.80	24.68	24.68	MR
Inbred Lines					
1	CI-4 (Geetha)	18.28	21.18	21.18	MR
2	HS-2	14.04	16.08	16.08	MR
3	CM-202	16.73	19.41	19.41	MR
4	9202B	17.21	20.00	20.00	MR
5	9208B	15.97	18.21	18.21	MR
6	MI-39	19.71	20.87	20.87	MR
7	CAH-1457	17.09	19.13	19.13	MR
8	CAL-1435	22.57	24.41	24.41	MR
9	HTMR-1401-1	17.66	21.09	21.09	MR
10	CML-286	3.49	14.18	14.18	MR
11	CML-451	19.87	22.25	22.25	MR
12	E-1 (Bidar)	18.97	21.50	21.50	MR
13	E-2 (Bidar)	17.13	19.29	19.29	MR
14	E-3 (Bidar)	16.20	18.26	18.26	MR
15	E1 CIMMYT B’gudi	4.03	16.53	16.53	MR
16	E2 CIMMYT B’gudi	14.84	16.89	16.89	MR
17	E4 CIMMYT B’gudi	20.51	23.13	23.13	MR
18	CIMMYT-3	28.79	30.05	30.05	MS
19	CIMMYT-4	20.12	23.15	23.15	MR
20	CIMMYT-5	19.60	18.03	19.60	MR
21	CIMMYT-6	18.99	22.03	22.03	MR
22	CIMMYT-7	20.54	24.80	24.80	MR
23	CIMMYT-10	20.99	24.94	24.94	MR
24	CIMMYT-12	25.03	30.75	30.75	MS
25	CIMMYT-13	22.50	25.17	25.17	MR
26	CIMMYT-16	28.81	31.78	31.78	MS
27	CIMMYT-17	34.63	31.13	34.63	MS
28	CIMMYT-19	28.94	22.12	28.94	MS
29	CIMMYT-21	19.66	23.17	23.17	MR
30	CIMMYT-22	19.39	22.48	22.48	MR
31	CIMMYT-23	23.16	26.19	26.19	MS
32	CIMMYT-24	27.12	32.37	32.37	MS
33	CIMMYT-25	27.78	30.39	30.39	MS
34	CIMMYT-26	18.27	20.45	20.45	MR
35	CIMMYT-27	19.54	21.71	21.71	MR
36	CIMMYT-28	18.24	20.07	20.07	MR
37	CIMMYT-30	22.43	25.78	25.78	MS
38	CIMMYT-32	22.74	26.32	26.32	MS
39	CIMMYT-33	11.82	12.98	12.98	MR
40	CIMMYT-35	21.61	24.95	24.95	MR
41	CIMMYT-36	19.27	21.48	21.48	MR
42	CIMMYT-38	25.30	30.65	30.65	MS
43	CIMMYT-39	22.52	25.33	25.33	MR
44	CIMMYT-40	20.37	24.15	24.15	MR
45	CIMMYT-42	18.27	20.49	20.49	MR
46	CIMMYT-43	22.35	25.00	25.00	MR
47	CIMMYT-44	18.15	20.14	20.14	MR
48	CIMMYT-45	22.47	25.22	25.22	MR
49	CIMMYT-46	24.76	30.43	30.43	MS
50	CIMMYT-47	23.52	27.85	27.85	MS
51	CIMMYT-48	25.18	31.55	31.55	MS
52	CIMMYT-49	22.86	25.59	25.59	MR
53	CIMMYT-51	24.49	21.16	24.49	MR
54	CIMMYT-52	29.12	34.55	34.55	MS
55	CIMMYT-54	28.45	25.98	28.45	MS
56	CIMMYT-55	21.03	22.81	22.81	MR
57	CIMMYT-56	21.24	25.78	25.78	MR
58	CUASR-I	25.56	26.74	26.74	MS
59	CUASR-II	22.35	26.10	26.10	MS
60	CUASR-III	19.59	22.19	22.19	MR
61	CM-111	23.76	26.55	26.55	MS
62	HS-7	21.74	25.24	25.24	MR
63	HS-17	23.56	21.41	23.56	MR

64	KDMI-15	19.01	21.81	21.81	MR
65	HS-4	17.58	19.19	19.19	MR
66	CML-446	20.59	22.84	22.84	MR
67	HS-10	21.24	26.61	26.61	MS

Table 3: Screening of breeding lines of maize against *F. verticilloides*, mycotoxin producing fungi

Sl. No.	**Maize Germplasm**	**Seed Infection (%)**		**Seed Infection (%)**	**Reaction**
		2017	**2018**		
UASR Hybrids					
1	CAH-1437	16.87	23.38	23.38	MR
2	CAH-1525	16.24	22.21	22.21	MR
3	CAH-1526	15.17	20.64	20.64	MR
4	CAH-1545	17.97	23.95	23.95	MR
5	CP-818	14.34	19.16	19.16	MR
6	900M	19.06	18.03	19.06	MR
7	E-1	21.11	27.44	27.44	MS
8	E-2	15.12	20.88	20.88	MR
9	E-3	17.61	23.82	23.82	MR
10	NK-6240	15.78	22.03	22.03	MR
11	Z-788-5	14.81	21.20	21.20	MR
12	Z-788-19	20.55	32.48	32.48	MS
13	Z-813-8	13.74	19.28	19.28	MR
CIMMYT Hybrids					
1	Z979-31	26.52	22.89	26.52	MS
2	Z979-53	5.49	17.96	17.96	MR
3	Z979-45	21.83	28.13	28.13	MS
4	Z979-22	15.46	20.63	20.63	MR
5	Z979-50	4.93	16.10	16.10	MR
6	Z979-29	6.36	17.79	17.79	MR
7	Z979-6	14.20	20.28	20.28	MR
8	Z979-51	5.44	22.94	22.94	MR
9	Z979-49	15.44	21.88	21.88	MR
10	Z979-7	15.30	25.13	25.13	MR
11	Z979-46	17.61	28.99	28.99	MS
12	Z979-28	19.04	24.90	24.90	MR
13	LC-3	15.66	21.29	21.29	MR
14	Z979-8	22.53	28.23	28.23	MS
15	Z979-2	20.17	26.15	26.15	MS
16	Z979-30	18.47	26.70	26.70	MS
17	LC-1	20.56	26.78	26.78	MS
18	Z979-40	31.61	36.49	36.49	MS
19	Z979-39	18.01	25.07	25.07	MR
20	Z979-16	20.11	31.31	31.31	MS
21	Z979-48	17.57	23.88	23.88	MR
22	Z979-23	4.88	19.71	19.71	MR
23	Z979-38	18.14	24.30	24.30	MR
24	Z979-1	20.67	27.30	27.30	MS
25	Z979-37	6.64	21.21	21.21	MR
26	Z979-36	24.15	31.89	31.89	MS
27	Z979-20	6.91	18.55	18.55	MR
28	Z979-27	13.06	19.11	19.11	MR
29	Z979-17	5.58	15.64	15.64	MR
30	Z979-15	17.78	28.89	28.89	MS
31	Z979-04	23.02	34.17	34.17	MS
32	Z979-34	17.51	16.01	17.51	MR
33	Z979-19	19.09	29.86	29.86	MS
34	Z979-10	17.51	23.03	23.03	MR
35	Z979-21	17.24	26.01	26.01	MS
36	Z979-25	15.24	20.71	20.71	MR
37	LC-2	14.98	21.27	21.27	MR
38	Z979-13	11.90	21.47	21.47	MR
39	Z979-41	17.82	24.47	24.47	MR
40	Z979-47	16.64	26.94	26.94	MS
41	Z979-24	15.65	24.25	24.25	MR
42	Z979-9	28.09	34.58	34.58	MS
43	Z979-35	19.40	18.82	19.40	MR
44	Z979-1	14.29	24.13	24.13	MR
45	Z979-54	15.99	14.88	15.99	MR

Inbred Lines					
1	CI-4 (Geetha)	24.49	30.83	30.83	MS
2	HS-2	19.95	26.00	26.00	MS
3	CM-202	13.30	18.25	18.25	MR
4	9202B	21.73	28.06	28.06	MS
5	9208B	17.55	23.34	23.34	MR
6	MI-39	18.16	23.88	23.88	MR
7	CAH-1457	11.75	15.70	15.70	MR
8	CAL-1435	14.15	20.59	20.59	MR
9	HTMR-1401-1	19.88	26.33	26.33	MS
10	CML-286	6.43	11.71	11.71	MR
11	CML-451	17.94	24.16	24.16	MR
12	E-1 (Bidar)	14.82	21.50	21.50	MR
13	E-2 (Bidar)	17.13	23.30	23.30	MR
14	E-3 (Bidar)	13.65	22.67	22.67	MR
15	E1 CIMMYT B’gudi	7.37	16.94	16.94	MR
16	E2 CIMMYT B’gudi	18.22	24.38	24.38	MR
17	E4 CIMMYT B’gudi	25.18	31.61	31.61	MS
18	CIMMYT-3	19.51	26.41	26.41	MS
19	CIMMYT-4	16.45	23.41	23.41	MR
20	CIMMYT-5	17.53	24.63	24.63	MR
21	CIMMYT-6	17.69	24.01	24.01	MR
22	CIMMYT-7	17.96	29.84	29.84	MS
23	CIMMYT-10	17.63	26.82	26.82	MS
24	CIMMYT-12	20.67	32.63	32.63	MS
25	CIMMYT-13	20.93	24.90	24.90	MR
26	CIMMYT-16	24.18	29.67	29.67	MS
27	CIMMYT-17	22.35	31.98	31.98	MS
28	CIMMYT-19	18.59	19.35	19.35	MR
29	CIMMYT-21	22.47	32.41	32.41	MS
30	CIMMYT-22	24.76	32.62	32.62	MS
31	CIMMYT-23	23.52	37.90	37.90	MS
32	CIMMYT-24	25.18	38.37	38.37	MS
33	CIMMYT-25	22.01	32.23	32.23	MS
34	CIMMYT-26	15.00	13.27	15.00	MR
35	CIMMYT-27	18.42	27.04	27.04	MS
36	CIMMYT-28	18.18	26.40	26.40	MS
37	CIMMYT-30	19.59	30.17	30.17	MS
38	CIMMYT-32	21.29	20.09	21.19	MR
39	CIMMYT-33	21.74	34.49	34.49	MS
40	CIMMYT-35	23.56	34.70	34.70	MS
41	CIMMYT-36	19.01	30.58	30.58	MS
42	CIMMYT-38	17.30	15.99	15.99	MR
43	CIMMYT-39	18.55	29.34	29.34	MS
44	CIMMYT-40	21.24	40.35	40.35	MS
45	CIMMYT-42	18.10	27.76	27.76	MS
46	CIMMYT-43	21.15	31.63	31.63	MS
47	CIMMYT-44	20.47	19.02	20.47	MR
48	CIMMYT-45	34.47	31.47	34.47	MS
49	CIMMYT-46	24.76	34.67	34.67	MS
50	CIMMYT-47	23.52	32.20	32.20	MS
51	CIMMYT-48	20.21	30.76	30.76	MS
52	CIMMYT-49	20.30	25.13	25.13	MR
53	CIMMYT-51	23.89	30.80	30.80	MS
54	CIMMYT-52	24.72	31.75	31.75	MS
55	CIMMYT-54	28.79	34.18	34.18	MS
56	CIMMYT-55	20.12	26.84	26.84	MS
57	CIMMYT-56	19.60	26.69	26.69	MS
58	CUASR-I	17.66	28.32	28.32	MS
59	CUASR-II	20.54	29.56	29.56	MS
60	CUASR-III	20.99	29.29	29.29	MS
61	CM-111	23.19	32.08	32.08	MS
62	HS-7	22.50	31.81	31.81	MS
63	HS-17	28.81	37.65	37.65	MS
64	KDMI-15	27.09	36.85	36.85	MS
65	HS-4	28.94	35.67	35.67	MS
66	CML-446	19.53	18.27	19.53	MR
67	HS-10	24.84	36.74	36.74	MS

Table 4: Reaction of maize germplasm against *A. flavus* contamination

Sl. No.	Reaction	Germplasm (2017-18)	Germplasm (2018-19)	Germplasm (Pooled)
1	Highly Resistant	Z979-31, Z979-7 and CML-286 (3 germplasm)	None	None
2	Resistant	Z979-22, Z979-29, Z979-2, LC-1, Z979-48, Z979-23, Z979-37, Z979-38, Z979-13, Z979-24 and E1 CIMMYT B’gudi (11 germplasm)	Z979-38	Z979-38
3	Moderately Resistant	CAH-1437, CAH-1525, CAH-1526, CAH-1545, CP-818, 900M, E-1, E-2, E-3, NK-6240, Z-788-5, Z-788-19, Z-813-8, Z979-53, Z979-45, Z979-50, Z979-6, Z979-51, Z979-49, Z979-46, Z979-28, LC-3, Z979-8, Z979-40, Z979-39, Z979-16, Z979-48, Z979-1, Z979-36, Z979-20, Z979-27, Z979-17, Z979-15, Z979-04, Z979-34, Z979-10, Z979-21, Z979-25, LC-2, Z979-41, Z979-47, Z979-9, Z979-35, Z979-1, Z979-54, CI-4 (Geetha), HS-2, CM-202, 9202B, 9208B, MI-39, CAH-1457, CAL-1435, HTMR-1401-1, CML-451, E-1 (Bidar), E-2 (Bidar), E-3 (Bidar), E2 CIMMYT B’gudi, E4 CIMMYT B’gudi, CIMMYT-4, CIMMYT-5, CIMMYT-6, CIMMYT-7, CIMMYT-10, CIMMYT-12, CIMMYT-13, CIMMYT-21, CIMMYT-22, CIMMYT-23, CIMMYT-26, CIMMYT-27, CIMMYT-28, CIMMYT-30, CIMMYT-32, CIMMYT-33, CIMMYT-35, CIMMYT-36, CIMMYT-38, CIMMYT-39, CIMMYT-40, CIMMYT-42, CIMMYT-43, CIMMYT-44, CIMMYT-45, CIMMYT-46, CIMMYT-47, CIMMYT-48, CIMMYT-49, CIMMYT-51, CIMMYT-55, CIMMYT-56, CUASR-I, CUASR-II, CUASR-III, CM-111, HS-7, HS-17, KDMI-15, HS-4, CML-446 and HS-10 (102 germplasm)	CAH-1437, CAH-1525, CAH-1526, CAH-1545, CP-818, 900M, E-1, E-2, E-3, NK-6240, Z-788-5, Z-788-19, Z-813-8, Z979-31, Z979-53, Z979-45, Z979-22, Z979-50, Z979-29, Z979-6, Z979-51, Z979-49, Z979-7, Z979-46, Z979-28, LC-3, Z979-8, Z979-2, LC-1, Z979-40, Z979-16, Z979-48, Z979-23, Z979-1, Z979-37, Z979-36, Z979-20, Z979-27, Z979-17, Z979-15, Z979-34, Z979-19, Z979-10, Z979-21, LC-2, Z979-13, Z979-41, Z979-47, Z979-24, Z979-9 Z979-35, Z979-1, Z979-54, CI-4 (Geetha), HS-2, CM-202, 9202B, 9208B, MI-39, CAH-1457, CAL-1435, HTMR-1401-1, CML-286, CML-451, E-1 (Bidar), E-2 (Bidar), E-3 (Bidar), E1 CIMMYT B’gudi, E2 CIMMYT B’gudi, E4 CIMMYT B’gudi, CIMMYT-4, CIMMYT-5, CIMMYT-6, CIMMYT-7, CIMMYT-10, CIMMYT-13 CIMMYT-19, CIMMYT-21 CIMMYT-22, CIMMYT-26 CIMMYT-27, CIMMYT-28 CIMMYT-30, CIMMYT-33 CIMMYT-35, CIMMYT-36 CIMMYT-39, CIMMYT-40 CIMMYT-42, CIMMYT-43 CIMMYT-44, CIMMYT-45 CIMMYT-49, CIMMYT-51 CIMMYT-54, CIMMYT-55 CIMMYT-56, CUASR-III HS-7, HS-17, KDMI-15, HS-4 and CML-446 (103 germplasm)	CAH-1437, CAH-1525, CAH-1526, CAH-1545, CP-818, 900M, E-1, E-2, E-3, NK-6240, Z-788-5, Z-788-19, Z-813-8, Z979-31, Z979-53, Z979-45, Z979-22, Z979-50, Z979-29, Z979-6, Z979-51, Z979-49, Z979-7, Z979-46, Z979-28, LC-3, Z979-8, Z979-2, LC-1, Z979-40, Z979-16, Z979-48, Z979-23, Z979-1, Z979-37, Z979-36, Z979-20, Z979-27, Z979-17, Z979-15, Z979-34, Z979-10, Z979-21, LC-2, Z979-13, Z979-41, Z979-47, Z979-24, Z979-9 Z979-35, Z979-1, Z979-54, CI-4 (Geetha), HS-2, CM-202, 9202B, 9208B, MI-39, CAH-1457, CAL-1435, HTMR-1401-1, CML-286, CML-451, E-1 (Bidar), E-2 (Bidar), E-3 (Bidar), E1 CIMMYT B’gudi, E2 CIMMYT B’gudi, E4 CIMMYT B’gudi, CIMMYT-4, CIMMYT-5, CIMMYT-6, CIMMYT-7, CIMMYT-10, CIMMYT-13 CIMMYT-21, CIMMYT-22, CIMMYT-26, CIMMYT-27, CIMMYT-28, CIMMYT-33, CIMMYT-35, CIMMYT-36, CIMMYT-39, CIMMYT-40, CIMMYT-42, CIMMYT-43, CIMMYT-44, CIMMYT-45, CIMMYT-49, CIMMYT-51, CIMMYT-55, CIMMYT-56, CUASR-III, HS-7, HS-17, KDMI-15, HS-4 and CML-446 (99 germplasm)
4	Moderately Susceptible	Z979-19, CIMMYT-3, CIMMYT-16, CIMMYT-17, CIMMYT-19, CIMMYT-24, CIMMYT-25, CIMMYT-52 and CIMMYT-54 (9 germplasm)	Z979-30, Z979-39, Z979-04, Z979-25, CIMMYT-3, CIMMYT-12 CIMMYT-16, CIMMYT-17 CIMMYT-32, CIMMYT-23 CIMMYT-24, CIMMYT-25 CIMMYT-38, CIMMYT-46 CIMMYT-47, CIMMYT-48 CIMMYT-52, CUASR-I, CUASR-II CM-111, HS-10 (21 germplasm)	Z979-30, Z979-39, Z979-04, Z979-25, Z979-19, CIMMYT-3, CIMMYT-12, CIMMYT-16, CIMMYT-17, CIMMYT-32, CIMMYT-23, CIMMYT-24, CIMMYT-25, CIMMYT-38, CIMMYT-46, CIMMYT-47, CIMMYT-48, CIMMYT-19, CIMMYT-30, CIMMYT-54, CIMMYT-52, CUASR-I, CUASR-II CM-111, HS-10 (25 germplasm)

Table 5: Reaction of maize germplasm against *F. verticilloides* contamination

Sl. No.	Reaction	Germplasm (2017-18)	Germplasm (2018-19)	Germplasm (Pooled)
1	Highly Resistant	None	None	None
2	Resistant	Z979-53, Z979-50, Z979-51, Z979-29, Z979-23, Z979-37, Z979-20, Z979-17 CML-286 and E1 CIMMYT B’gudi (10 germplasm)	None	None
3	Moderately Resistant	CAH-1437, CAH-1525, CAH-1526, CAH-1545, CP-818, 900M, E-1, E-2, E-3, NK-6240, Z-788-5, Z-788-19, Z-813-8 , Z979-45, Z979-22, Z979-6, Z979-49, Z979-7, Z979-46 , Z979-28, LC-3, Z979-8, Z979-2, Z979-30, LC-1, Z979-39, Z979-16, Z979-48, Z979-38, Z979-1, Z979-36, Z979-27, Z979-15, Z979-04, Z979-34, Z979-19, Z979-10, Z979-21, Z979-25, LC-2, Z979-13, Z979-41, Z979-47, Z979-24, Z979-35,	CAH-1437, CAH-1525, CAH-1526, CAH-1545, CP-818, 900M, E-2, E-3, NK-6240, Z-788-5, Z-813-8, Z979-31, Z979-53, Z979-22, Z979-50, Z979-29, Z979-6, Z979-51, Z979-49, Z979-7, Z979-28, LC-3, Z979-39, Z979-48, Z979-23, Z979-38, Z979-37, Z979-20, Z979-27, Z979-17, Z979-34, Z979-10,	CAH-1437, CAH-1525, CAH-1526, CAH-1545, CP-818, 900M, E-1, E-2, E-3, NK-6240, Z-788-5, Z-788-19, Z-813-8, Z979-53, Z979-22, Z979-50 Z979-29, Z979-6, Z979-51, Z979-49, Z979-7, Z979-28, LC-3, Z979-39, Z979-48, Z979-23, Z979-38, Z979-37, Z979-20, Z979-27, Z979-17, Z979-34, Z979-10, Z979-25, LC-2, Z979-13, Z979-41,

		Z979-1, Z979-54, CI-4 (Geetha), HS-2, CM-202, 9202B, 9208B, MI-39, CAH-1457, CAL-1435, HTMR-1401-1, CML-451, E-1 (Bidar), E-2 (Bidar), E-3 (Bidar), E2 CIMMYT B'gudi, CIMMYT-3, E4 CIMMYT B'gudi, CIMMYT-4, CIMMYT-5, CIMMYT-6, CIMMYT-7, CIMMYT-10, CIMMYT-12, CIMMYT-13, CIMMYT-16, CIMMYT-17, CIMMYT-19, CIMMYT-21, CIMMYT-22, CIMMYT-23, CIMMYT-24, CIMMYT-25, CIMMYT-26, CIMMYT-27, CIMMYT-28, CIMMYT-30, CIMMYT-32, CIMMYT-33, CIMMYT-35, CIMMYT-36, CIMMYT-38, CIMMYT-39, CIMMYT-40, CIMMYT-42, CIMMYT-43, CIMMYT-44, CIMMYT-46, CIMMYT-47, CIMMYT-48, CIMMYT-49, CIMMYT-51, CIMMYT-52, CIMMYT-55, CIMMYT-56, CUASR-I, CUASR-II, CUASR-III, CM-111, HS-7, CML-446 and HS-10 (107 germplasm)	Z979-25, LC-2, Z979-13, Z979-41, Z979-24, Z979-35, Z979-1, Z979-54, CM-202, 9208B, MI-39, CAH-1457, CAL-1435, CML-286, CML-451, E-1 (Bidar), E-2 (Bidar), E-3 (Bidar), E1 CIMMYT B'gudi, CIMMYT-5, E2 CIMMYT B'gudi, CIMMYT-4, CIMMYT-6, CIMMYT-13, CIMMYT-19, CIMMYT-26, CIMMYT-32, CIMMYT-38, CIMMYT-44, CIMMYT-49 and CML-446 (63 germplasm)	Z979-24, Z979-35, Z979-1, Z979-54, CM-202, ,9208B, MI-39, CAH-1457, CAL-1435, CML-286, CML-451, E-1 (Bidar), E-2 (Bidar), E-3 (Bidar), E1 CIMMYT B'gudi, CIMMYT-4 E2 CIMMYT B'gudi, CIMMYT-5 CIMMYT-6, CIMMYT-13, CIMMYT-19, CIMMYT-26, CIMMYT-32, CIMMYT-38, CIMMYT-44, CIMMYT-49 and CML-446 (59 germplasm)
4	Moderately Susceptible	Z979-31, Z979-40, Z979-9, HS-17, CIMMYT-45, CIMMYT-54, HS-4 and KDMI-15 (8 germplasm)	E-1, Z-788-19, Z979-45, Z979-46, Z979-8, Z979-2, Z979-30, LC-1, Z979-40, Z979-16, Z979-1, Z979-36, Z979-15, Z979-04, Z979-19, Z979-21, Z979-47, Z979-9, CI-4 (Geetha), HS-2, 9202B, HTMR-1401-1, E4 CIMMYT B'gudi, CIMMYT-3, CIMMYT-7, CIMMYT-10, CIMMYT-12, CIMMYT-16, CIMMYT-17, CIMMYT-21, CIMMYT-22, CIMMYT-23, CIMMYT-24, CIMMYT-25, CIMMYT-27, CIMMYT-28, CIMMYT-30, CIMMYT-33, CIMMYT-35, CIMMYT-36, CIMMYT-39, CIMMYT-40, CIMMYT-42, CIMMYT-43, CIMMYT-45, CIMMYT-46, CIMMYT-47, CIMMYT-48, CIMMYT-51, CIMMYT-52, CIMMYT-54, CIMMYT-55, CIMMYT-56, CUASR-I, CUASR-II, CUASR-III, CM-111, HS-7, HS-17, KDMI-15, HS-4 and HS-10 (62 germplasm)	Z979-31, Z979-45, Z979-46 , Z979-8 Z979-2, Z979-30, LC-1, Z979-40, Z979-16, Z979-1, Z979-36, Z979-15, Z979-04, Z979-19, Z979-21, Z979-47, Z979-9, CI-4 (Geetha), HS-2, 9202B, HTMR-1401-1, E4 CIMMYT B'gudi, CIMMYT-3, CIMMYT-7, CIMMYT-10, CIMMYT-12, CIMMYT-16, CIMMYT-17, CIMMYT-21, CIMMYT-22, CIMMYT-23, CIMMYT-24, CIMMYT-25, CIMMYT-27, CIMMYT-28, CIMMYT-30, CIMMYT-33, CIMMYT-35, CIMMYT-36, CIMMYT-39, CIMMYT-40, CIMMYT-42, CIMMYT-43, CIMMYT-45, CIMMYT-46, CIMMYT-47, CIMMYT-48, CIMMYT-51, CIMMYT-52, CIMMYT-54, CIMMYT-55, CIMMYT-56, CUASR-I, CUASR-II, CUASR-III, CM-111, HS-7, HS-17, KDMI-15, HS-4 and HS-10 (66 germplasm)

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