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RESPONSES OF SPRING SOWN MAIZE (*ZEA MAYS* L.) GENOTYPES TO *ASPERGILLUS FLAVUS* INOCULATION: GRAIN YIELD AND QUALITY ATTRIBUTES

SADIA ZAFAR[12], NAEEM IQBAL[2*], MUHAMMAD ZULQURNAIN HAIDER[2], MUHAMMAD RAFIQUE[3]AND MUHAMMAD ALI[24]

[1]*Department of Botany, Division of Science and Technology, University of Education, Lahore 54770, Punjab, Pakistan*
[2]*Department of Botany, Government College University Faisalabad, Pakistan*
[3]*Ayub Agricultural Research Institute Faisalabad, Pakistan*
[4]*Quaid-e-AzamUniversity, Islamabad, Pakistan*
**Corresponding author's email: drnaeem@gcuf.edu.pk*

Abstract

Maize, the one of the world's most important cereals is susceptible to an opportunistic pathogen, *A. flavus*producing aflatoxins, which ultimately causing economic as well as human health risks. The insufficient understandings of maize resistance to the fungus have made the selection of resistant genotypes difficult for scientists and growers. A field trial was conducted to find out the host responses to *A. flavus* exposure in terms of grain yield and quality of maize during the spring season. Maize cob of fungal inoculated and non-inoculated plants were harvested manually and then dried under shade at room temperature. After drying of maize cob, yield and quality related attributed were investigated. The results revealed that non-inoculated maize plants had cob of greater weight than those of *A. flavus* inoculated maize plants, despite negligible record of visible symptoms of infection in all maize genotypes. The exposure of plants to *A. flavus* negatively affected the grains per cob, total grains weight/cob and protein, oil as well as carbohydrate contents of grains. Maize genotype, KSC-9663 produced higher value for cob length, diameter as well as weight and grains weight per cob. While, maize genotype, HC-9091 showed the lowest value for cob length, diameter and grains weight per cob. Based on the results of current studies, it can be concluded that *A. flavus* inoculation may have negative effects on grain yield and quality attributes of maize under climatic conditions that favor the fungus growth without producing visible symptoms of infection.

Key words: Cob yield attributes, Fungal infection, Maize grain, Grain oil.

Introduction

Pre-harvest infection of maize by fungal species and subsequent contamination of grains with toxins has been a continuous threat for food safety (Park & Liang, 1993; Windham & Willim, 2016; Chauke *et al*., 2018). Aflatoxins, the widely studied mycotoxins have the ability to suppress human and animal immune system, thereby negatively affecting growth and development in living organisms (Chauke *et al*., 2018). Considering the negative effects of these mycotoxins, many countries have established regulations for controlling aflatoxins contaminations in food and feed (Cleveland *et al.*, 2003; Betran & Isakeit, 2004; Anon., 2004). A desirable approach for reducing *Aspergillus* infection and/or aflatoxin production is the selection/development of resistant germplasm. Several natural sources of resistance have been identified in maize (van Loon *et al*., 2006). However, the genotype environment interactions and quantitative nature of the trait restrict the transfer of resistance into elite breeding lines (Warburton *et al*., 2011).

Maize, the one of the most important cereal crops in the world agricultural economy is used as food for the humans and fodder for animals (Basson *et al.*, 2018). Maize is prone to contamination of aflatoxins produced by *Aspergillus* (Castells *et al*., 2008; Misihairabgwi *et al*., 2017). The main strategy to overcome the problem of aflatoxins contamination is the identification of resistant germplasm of maize (Hawkins *et al*., 2015). Hence, the development of fungus resistant germplasm has been the subject of many studies throughout the world (Scott & Zummo, 1990, 1992; Williams & Windham, 2001; Williams *et al*., 2005; Williams & Windham, 2006; Windham & William, 2016).

*Aspergillus flavus*is generally considered a weak pathogen and may show sporadic infection between two growing seasons (Zummo & Scott, 1989; Windham *et al.*, 2005; Windham & Willim, 2016). Hence, to evaluate maize genotypes for resistance to *A. flavus*, scientists have developed inoculation techniques (William *et al*., 2005). In addition to choosing an appropriate inoculation technique, it is also necessary to use an isolate from the *A. flavus* group that produces adequate level of infection. For instance, *A. flavus* strain NRRL 3357 has been used in maize germplasm evaluation for aflatoxins resistance (William *et al*., 2005; Windham & William, 2016). Considerable evidence is available concerning the ability of this fungus to invade the crops and produce toxins, while still in the field (Pitt & Hocking, 2009). Aflatoxin's contamination of food grains have been a major concern for many countries of Asia, America and Africa (Bankole & Adebanjo, 2003). Many approaches, like detoxification of mycotoxins from grains, application of natural and synthetic chemicals and development of resistant varieties are being utilized to control fungal infection and aflatoxins contamination (Bankole & Adebanjo, 2003).

Our approach is to evaluate the potential of the genotype(s) with respect to resistance to *A. flavus* and its impact on grain yield and quality attributes grown maize. The study has been performed to find out the better maize genotype(s) in term of yield and quality with resistance against *A. flavus* contamination and to determine the effects of *A. flavus* inoculation on grain yield and quality of maize in open field conditions at pre-harvest time.

Material and Methods

Field experiment was carried out to find out a better maize genotype, resistant to *A. flavus* contamination and also produce better yield and better grain nutritional quality.

Maize genotypes: The experiment was conducted using commercially available 14 maize genotypes. Three varieties (Pearl, MALKA, and MMRI) and eleven hybrids (YH-1898, FH-949, HC-9091, HC-2040, K.S.C 9663, K.S.C 9618, R-2315, R-33334, R-3305, R-2207 and FH-1046) were obtained from Ayub Agricultural Research Institute, Maize & Millets Research Institute, Yousafwala, Sahiwal and Rafhan Maize Products, Faisalabad. Experiments were conducted in the field of Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan (latitude 31 ° 26´ N, longitude 73° 04´ E and 184 m) during spring 2017 in semi-arid conditions.

Field design: The experiment was conducted in total area of 525 m^2 in a randomized complete block design with five repeats. Plot size was 5.0 x 0.75m, row to row and plant to plant distances were 75 and 20 cm, respectively. Fertilizer (N: 297 kg/ha, P: 148 kg/ha and K: 124 kg/ha) were applied at the vegetative stage. All cultural and agronomic practices were followed accordingly, including hoeing, weeding, irrigation and insect management, etc.

Meteorological conditions during experiment time: The meteorological data, including temperature (24.8-40°C), rainfall (3.7-111mm) and relative humidity (32.2-77.6%) of growing time from February to July, was obtained from the climatic station (about 300m from field) Department of Climate Change, Ayub Agriculture Research Institute Faisalabad (Pakistan).

Preparation and application of fungal inoculum: Mycelia taken from *Aspergillus* infected grains were transferred with a sterile loop to the agar surface (PDA). All the plates were incubated at 28°C for five days. Conidia were washed from the grits using sterile distilled water containing 20 drops of Tween-20 per liter and centrifuged for 15 minutes at 3000*g*. Conidial concentration (1×10^4) was prepared by placing a drop of the stock conidial suspension on hemacytometer and counting conidia using a compound microscope. Dilutions were made from the stock of conidial suspension to obtain the desired concentrations (Windham & Williams, 2016). The fungal isolates were screened for ability to produce aflatoxins (Dyer & McCammon, 1994). The aflatoxin producer was identified as *A. flavus*by microscopic and macroscopic observation (Pitt *et al.*, 1983; Pitt *et al.*, 1992; Klich, 2002). The experimental area was divided into two equal parts. One group of maize plants received the inoculum of Aflatoxin producing fungi was applied into maize cob at grain filling stage.

Determination of yield and quality of maize: All non-inoculated and inoculated ears were harvested by hand at kernel maturity stage and data was recorded. The yield related attributes were recorded after final harvest of crop at maturity. The grain quality related attributes like protein, oil, as well as starch contents and grain moisture values were evaluated using kernel analyzer. Grain fiber, fat, ash as well as carbohydrate contents were also determined (Anon., 2000). The collected data were subjected to statistical analysis.

Results

The climatic conditions such as temperature (26.3-38.5ºC), relative humidity (45.9-73.4%) and rain fall (56-111mm) recorded at the time of *A. flavus* inoculation were favorable for fungal growth.

A. flavus inoculated and non-inoculated plants showed statistically significant differences for cob length (Table 1). As indicated by analysis of variance table, the maize genotypes differed highly significantly for cob length. In general the maize genotype, KSC-9663 produced higher cob length than all other genotypes, used in the study, followed by genotype Malka-16, R-2207, FH-1046, R-2315 and R3334 (Fig. 1a). The maize genotype HC-9091 produced the lowest value of cob length. Inoculated and non-inoculated plants showed statistically non-significant differences for cob diameter (Table 1). The maize genotype KSC-9663 produced highest cob diameter than all other genotypes, followed by genotype R-3305, FH-1046, Malka-16 and R3334 (Fig. 1b). The maize genotype Pearl and HC-9091 produced the lowest value for cob diameter. The cob weight of *A. flavus* inoculated and non-inoculated plants showed statistically highly significant differences (Table 1). The non-inoculated plants produced cob weight with 78% higher value than those of inoculated plants (Fig. 1c). The maize genotype, KSC-9663 produced highest cob weight followed by genotype, FH-1046. The maize genotype, YH-1898 followed by Pearl and MMRI produced the lowest value for cob weight.

The inoculated and non-inoculated plants showed statistically significant differences for grains/cob (Table 1). The non-inoculated plants showed 45% higher value for number of grains/cob than those of inoculated plants (Fig. 1d). The maize genotype, R-2315 produced highest grains per cob followed by genotype FH-1046 and KSC-9663. The maize genotype, R-3305 produced the lowest value followed by HC-9091 for grains per cob. The non-inoculated plants produced cob with high grain weight value (52% higher) than those of inoculated ones (Fig. 1e). Analysis of variance table showed that the maize genotypes had highly significant differences for total grains weight per cob. The maize genotype KSC-9663 produced highest value for grains weight per cob, followed by genotype R-2315 and FH-1046. The *A. flavus* inoculated and non-inoculated plants showed statistically highly significant differences for 100-grains weight (Table 1). Maize genotype, KSC-9663 produced highest 100-grains weight followed by genotypes, YH-1898 and R-3305 (Fig. 1f). The maize genotype FH-949 produced the lowest value followed by HC-2040 for 100 grains weight. The non-inoculated plants produced grains weight with high value (26% higher) than those of inoculated plants in all maize genotypes.

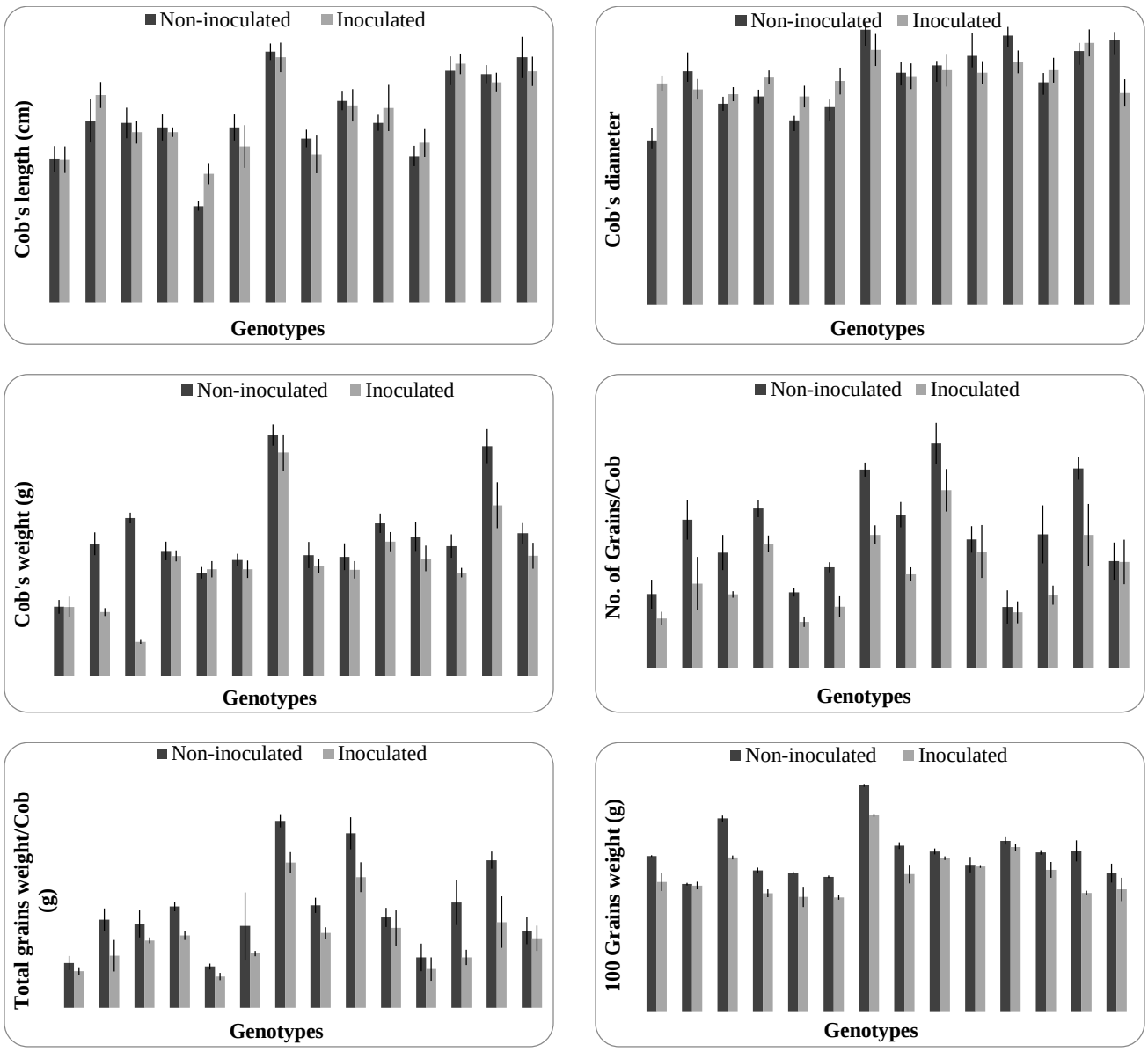

Fig. 1. Yield components of *A. flavus* inoculated and non-inoculated maize genotypes a) Cob length, b) Cob diameter, c) Cob weight, d) No. grains/cob, e) Total grains weight/cob and f) 100- grains weight (Data represent mean ± standard error).

The grain moisture contents values of *A. flavus* inoculated and non-inoculated plants showed statistically significant differences (Table 1). The maize genotype, R-3334 showed highest value for grain moisture contents than all other genotypes, followed by genotype R-2207 and FH-1046. The maize genotype, HC-2040 and HC-9091 produced the lowest value for grain moisture contents (Fig. 2a).

The non-inoculated plants produced 19% higher value for grain carbohydrate than those of *A. flavus* inoculated ones (Fig. 2b). The maize genotype, YH-1898 produced grains with highest carbohydrate levels than all other genotypes. The maize genotype, Malka-16 produced grains the lowest carbohydrate contents. Similarly, as far as the grain starch contents, the maize genotype YH-1898 showed highest andMalka-16 showed lowest levels of grain starch (Fig. 2c). The non-inoculated plants produced grains with 15% higher value of oil contents than those of inoculated ones (Fig. 2d).The maize genotype, MMRI showed the highest value for grain oil followed by genotype, FH-1046 and R-2207. The maize genotype, KSC-9663 produced the lowest value for grain oil contents. Similarly, the maize genotype, MMRI also showed higher grain fat contents than all other genotypes, followed by genotype, R-3334 (Fig. 2e).

Statistically non-significant differences were recorded for grain fiber contents of *A. flavus* inoculated and non-inoculated plants (Table 1). The maize genotype, YH-1898 showed higher grain fiber contents than all other genotypes, followed by genotype R-3305. The maize genotype Malka-16 produced the lowest fiber contents (Fig. 2f). The maize genotype, FH-1046 showed higher ash contents than all other genotypes, followed by genotype R-2207 and HC-2040 (Fig. 2g).

Maize genotype Pearl produced highest protein contents followed by genotype Malka-16. The maize genotype HC-2040 produced the lowest value for protein. The non-inoculated plants produced high value (1.33-13.3%) of protein than those of inoculated plants in all maize genotypes (Fig. 2h).

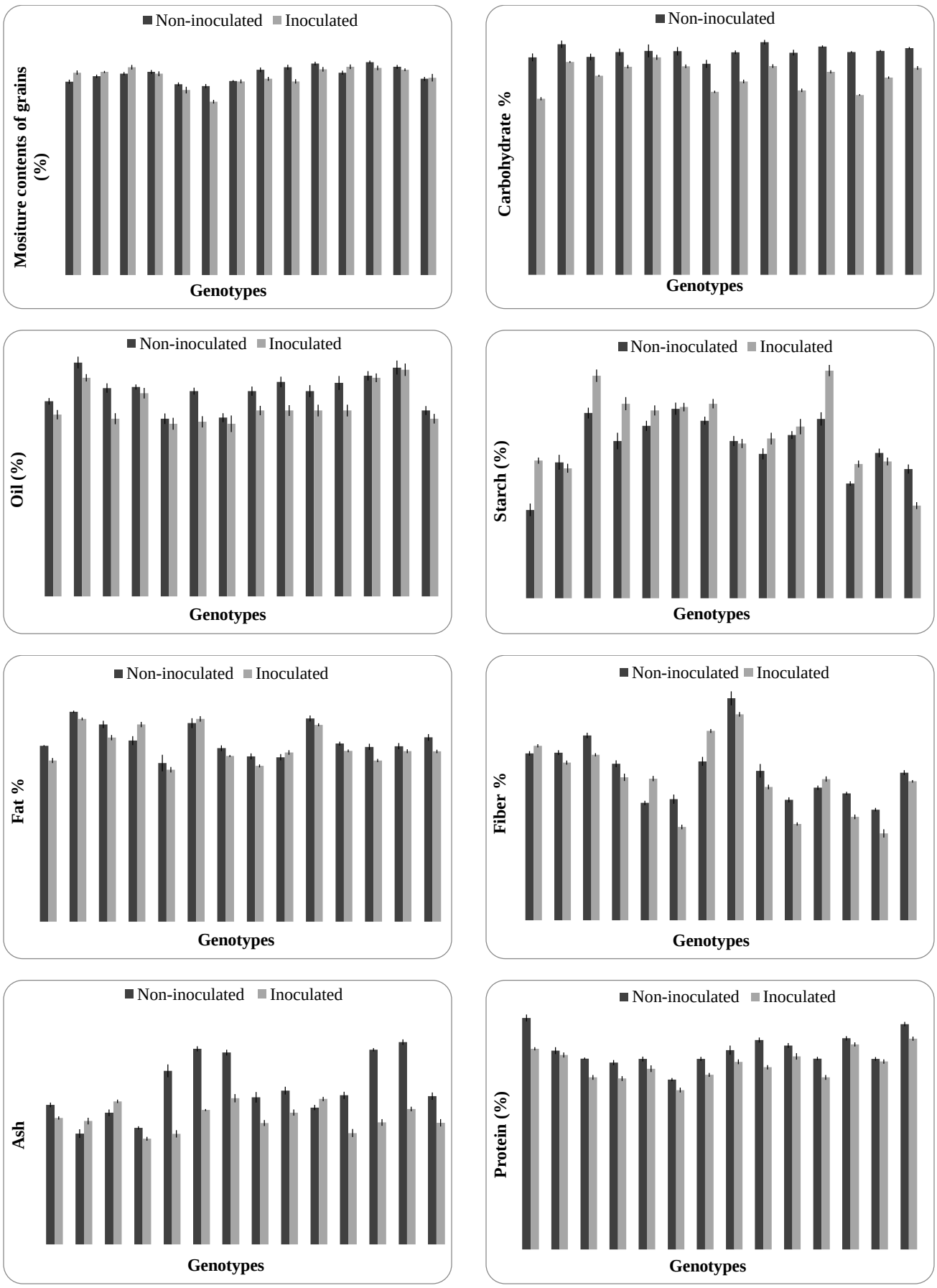

Fig. 2. Grain quality attributes of *A. flavus* inoculated and non-inoculated maize genotypes a) Moisture contents, b) Carbohydrate contents, c) Starch contents, d) Oil contents, e) Fat contents, f) Fiber contents, g) Ash contents, h) Protein contents (Data represent mean ± standard error).

Table 1. Mean square values from analysis of variance of data regarding gains yield components and quality parameters of different non-inoculated and inoculated maize genotypes.

	df	Cob length (cm)	Cob diameter (cm)	Cob weight (g)	Grain per cob	Total grains weight/cob (g)	100 grain weight (g)	Protein (mg/g)	Moisture	Starch (mg/g)	Oil (mg/g)	Fat (mg/g)	Fiber (mg/g)	Ash (mg/g)	Carbohydrate (mg/g)
Genotypes	13	113***	1.4***	18517***	113891***	14630***	220***	6.7***	1.9***	5.6***	1.1***	1.4***	2.1***	0.3***	93***
Treatments	1	0.8^{ns}	0.01^{ns}	28706***	332524***	26882***	559***	21***	0.7*	3.8***	3.2***	0.6***	0.4***	3.0***	2706***
Error	125	8.2	0.27	1025	9811	1077	8.33	0.16	0.12	0.17	0.07	0.04	0.03	0.03	8.3
Total	139														

* = p<0.05, ** = p<0.01, *** = p<0.001, ns = Non-significant, df= Degree of freedom

Table 2. Correlation between grain yield and quality parameters and maize genotypes.

	Cob's length (cm)	Cob's diameter	Cob's weight (g)	No. of grains/ Cob	Total grains weight/Cob (g)	100 Grains weight (g)	Protein (%)	Moisture (%)	Starch (%)	Oil (%)	Fat	Fiber	Ash	Carbohydrate
Genotype	0.22*	0.22**	0.04	-0.19*	0.14	-0.28**	-0.26**	0.02	0.09	-0.24**	0.12	-0.32**	0.00	0.64**
Cob's Length (cm)		0.39**	0.40**	0.32**	0.36**	0.20*	0.18*	0.23**	-0.21*	0.16	0.07	0.11	0.01	0.1
Cob's diameter			0.41**	0.21*	0.27**	0.18*	0.05	0.25**	0.08	0.15	0.01	0.09	0.00	0.06
Cob's Weight (g)				0.33**	0.39**	0.45**	0.08	0.02	0.08	0.06	0.03	0.07	0.06	0.07
No. of Grains/Cob					0.91**	0.24**	0.1	0.23**	0.11	0.26**	0.03	0.06	0.05	0.24**
Total grains weight/Cob (g)						0.41**	0.02	0.14	0.01	0.11	0.04	0.11	0.05	0.15
100 Grains Weight (g)							0.04	0.13	0.19*	0.06	0.04	0.31**	0.07	0.05
Protein (%)								0.30**	-0.74**	0.23**	-0.17*	0.11	0.06	0.21*
Mositure (%)									0.1	0.47**	0.01	0.02	0.03	0.03
Starch (%)										-0.28**	0.20*	0.01	0.07	0.13
Oil (%)											0.01	0.14	0.13	0.33**
Fat												0.11	0.1	0.13
Fiber													0.06	0.00
Ash														0.14

*, ** Significant at 0.05 and 0.01 levels of probability

Results (Table 2) showed that there was a negative correlation between genotype and carbohydrate. Cob length was positively and significantly correlated with cob diameter, cob weight, grains per cob and total grains weight per cob. Cob diameter showed moderate positive correlation for cob weight. Cob weight showed moderate positive correlation for grains per cob, total grains weight per cob and 100 grains weight. Grains per cob showed positive correlation for total grains weight per cob. Total grains weight per cob showed moderate positive correlation for 100-grains weight. 100= grains weight showed moderate positive correlation for fiber. Protein contents showed negative correlation for starch contents. Grain moisture contents were positively correlated for grain oil contents.

Discussion

The results of current study clearly revealed that the fungus inoculation did not produce prominent visible symptoms, though many of the attributes such as cob weight and grains per cob, total grains weight/cob, 100 grains weight, and grain protein contents, oil and carbohydrate of fungal inoculated plants showed lower values than non-inoculated ones. On the other hand, differential response to *A. flavus* inoculation among all the studied genotypes were recorded for cob length, cob diameter, and grain moisture, starch, fat, fiber and ash contents. Nonappearance of fungal infection symptoms in most of the maize genotypes had been attributed to non-favorable climatic conditions for fungal growth. The meteorological data represented in Table 1 indicated that weather conditions during current investigation were favorable for fungal growth. The previous studies had reported the appearance of the symptoms under almost similar meteorological conditions (Windham & Williams, 2016). The current findings that *A. flavus* grew well under high temperature and low moisture conditions could be confirmed by the results of many previous studies (Abbas *et al.*, 2007; Bellaloui *et al*., 2016; Windham & Williams, 2016). The other possibilities of nonappearance of fungus infection could be the use of resistant germplasm or diluted inoculums medium or the virulence level of the fungal strain itself. Many studies had indicated that less than optimum concentration of inoculums material may not be helpful in producing visible fungal infection. The fungal inoculated material used during the present investigation was in appropriate (1×10^4) concentration for producing disease pressure as reported by Windham and Williams (2016). Moreover, the nonappearance of infection on maize could be the used inoculation technique. Some reports had indicated that non-wounding inoculation technique was not appropriate for clear visible fungal infection (Windham & Williams, 2016). However, some other studies reported that the technique applied was very close to the natural infection process (reason for the selection of the technique) for production of aflatoxins (Windham & Williams, 2007) depending upon location, meteorological conditions and nature of maize germplasm (Williams & Windham, 2012; Windham & Williams, 2016).

The current investigation revealed that *A. flavus* inoculation did not affect the cob length, despite the climatic conditions were favorable for *A. flavus* growth. This could be explained by the fact that the inoculums

were applied at grain filling stage when the plants had almost attained their maximum cob length. The relatively higher cob length of maize genotype, KSC-9663 indicated its greater potential towards better yield, as reported by Rani *et al.,* (2017). The positive correlation of cob length with cob diameter, cob weight, grains per cob as recorded during current investigation had also been reported by previous studies (Afarinesh *et al*., 2005; Wang *et al.*, 2007). Hence, this could be considered as selection criteria for higher grain yield potential in maize genotypes (Rani *et al.*, 2017).

Though, the maize genotype, FH-949 showed relatively higher values than those of all other genotypes for most of the parameters recorded during present investigation; the 100-grain weight was amongst the lowest. The relatively lower grain weight production by maize genotype FH-949 than those of all other genotypes might be due to its genetic makeup (Grzesiak *et al*., 2007). The relatively higher value for cob length, cob diameter, cob weight, grains weight per cob and 100 grains weight of genotype, KSC-9663 suggested its higher production potential as compared to other genotypes used in current study. Similar relationship among higher yield potential and the cob weight, cob length and cob diameter, was reported by many other scientists working on *A. flavus* and plants interactions (Afarinesh *et al.*, 2005; Grzesiak*et al*., 2007). The less reduction in grains per cob, total grains weight per cob and 100 grains weight in resistant genotypes after treatment with *A. flavus* and positive correlation among all above mentioned attributes indicated resistance and higher yield potential of these genotypes (Ali & Ahsan, 2015). The findings of current investigation regarding positive correlations between yield and yield-related components of maize had also been reported by Ilker (2011), Hasyan *et al.,* (2012), Kumar *et al.,* (2014) and Karasu *et al*., (2015).

The grain protein contents (8.8-12%) recorded during current investigation had also been reported in almost similar range by Ijabadeniyi & Adebolu (2005) and Ullah *et al*., (2010) in maize. The maize genotype, HC-2040 produced the lowest value for protein and moisture contents. The values of grain fiber contents (1.22-2.89%) were also in the range as reported by previous studies in maize (Ijabadeniyi & Adebolu, 2005; Ullah *et al.*, 2010). Similarly, many scientists (Ullah *et al*., 2010) had reported approximately same level of grain ash contents in maize as recorded during current investigation (1.1-2.02%). On the other hand, Ijabadeniyi & Adebolu (2005) and Ullah *et al*., (2010) reported slightly low carbohydrate contents in maize grains as recorded during current investigation. The level of grain moisture (9.5-11.7%) recorded during current investigation had also been revealed by many previous studies (Dorsey-Redding *et al.,* 1990; Ullah *et al*., 2010). Maize genotype R-3334 showed highest moisture contents while maize genotype YH-1898 showed highest starch, fiber, ash and carbohydrate than all other maize genotypes in present investigation. On the other hand maize genotype Malka-16 produced the lowest starch, fiber, ash and carbohydrate. The positive correlation of grain oil contents with carbohydrate might have favored the growth of *A. flavus*asBellaloui *et al*., (2016) described the similar relation and the enhanced fungus growth. Many other studies also suggested that the carbohydrate contents had important role in *A. flavus* growth and aflatoxin biosynthesis (Ellis *et al*., 1991).

Keeping in view the above discussion, it is concluded that non-wounding *A. flauvs* inoculum may affect adversely the yield and quality of maize genotypes without appearance of symptoms on the corns. Furthermore, the process of *A. flavus* infection and resulting aflatoxin contamination in the grains is a multifaceted issue demanding the integrated approaches to overcome this problem without compromising the crop production as well as human health.

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