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Effects of *Aspergillus flavus* inoculation on spring Maize (*Zea mays* L.) grain yield

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Abstract

Maize (*Zea mays* L.) is a staple crop and widely consumed over the globe, but its production is very challenging due to its contamination by *Aspergillus species*. *Aspergillus flavus* is the most dominant fungi that produce aflatoxin in many major crops. Aflatoxin producing fungi mostly attack during the development of crops and induce infection. Present study was aimed to investigate the effects of *A. flavus* inoculation on grain yield of maize genotypes in open field environments at pre-harvest time. Fourteen maize genotypes were treated with *A. flavus* inoculum by non-wounding method (inoculated through cob silk), at grain filling stage and yield related parameters were recorded. Results indicated that maize genotype R-3305 produced higher values for cob length and number of grains per cob. Maize genotype FH-949 produced higher values for cob diameter and cob weight. Maize genotype FH-1046 produced higher value for total grains weight per cob and KSC-9663 produced higher values for 100 grains weight as compared to all other genotypes.HC-2040 produced lower values for yield related parameters as compared to all other genotypes. Based on the results of current work, it can be concluded that *A. flavus* inoculation affected the grain yield related parameters. The maize genotype, FH-949 and FH-1046produced better grain yield as compared to all other genotypes. Thus *A. flavus* inoculation affected the grain yield related component and had a pronounced role in the reduction of maize production.

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Introduction

Maize having a major role in economy of numerous countries (Khan *et al.*, 2015) is consumed as food for human and feed for livestock as well as poultry (Shakoor *et al.*, 2017). Maize is important source of proteins, carbohydrates, minerals and antioxidants which are essential components of daily nutrition and due to these qualities it has got great attention (Kuhnen *et al.*, 2010; Hiran *et al.*, 2016; Ogbaga *et al.*, 2017). Focusing the importance of maize in economic, food, feed and biofuel, there is intensive requirement to understand that how crop tolerates stress and reduce its effects that cause reduction in crop production (Ogbaga *et al.*, 2017).

Aspergillus genus is ubiquitous and is found in air, soil and in debris (Maina *et al.*, 2016). Numerous factors like moisture, drought stress, high temperature and delayed harvesting favour the infection of *Aspergillus species* and aflatoxin contamination (Atanda *et al.*, 2011; Maina *et al.*, 2016). Aflatoxin producing fungi infected the maize at different stages including pre-harvest, harvest and post-harvest stage and disturb the country's economy (Atanda *et al.*, 2011), thus maize production is very challenging due to the colonization by *Aspergillus species* (Wagacha and Muthomi, 2008).

A. flavus is most dominant fungi that produce aflatoxin in many major crops. Aflatoxigenic fungi mostly attack during development of crop and caused pre-harvest aflatoxin contamination, high temperature and other stresses favour its infection and aflatoxin contamination (Farfan *et al.*, 2015).

Climate change and its impacts have become more and more prominent from past few decades (Patt and Schroter, 2008). Climate change projections propose a more changing climate with higher susceptibilities in developing countries. Pakistan is considered as the most vulnerable country with respect to climate change because it has limited resources and geographical pattern (Schilling *et al.*, 2013). Pakistan is already facing the severity of climate change events like extreme temperature, drought and floods which ultimately resulted in high risk of infections and diseases (Smit and Skinner, 2002).

Aflatoxin contamination in the maize plant is very serious agricultural problem especially under high temperature and drought (Fountain *et al.*, 2014). Consumption of food and feed that is contaminated with aflatoxin, has been shown to affect child development, increase death rate in farm animals and liver cancer in human beings, or even cause aflatoxicosis and death in rare cases (Probst *et al.*, 2010). It was estimated that over 55 million people worldwide are exposed to aflatoxins at unhealthy levels (Liu and Wu, 2010). Due to these health risks, several countries have developed the strict rules regarding the permissible levels of aflatoxin in food and feed (Wu and Munkvold, 2008).

Plants have numerous host defense mechanisms that provide protection to the cell against stresses (Gong *et al.*, 2001; Foyer and Noctor, 2003; Pastori *et al.*, 2003; Wahid *et al.*, 2007). Plant susceptibility to aflatoxin is very complex feature and complete source of maize resistance is not known (Mayfield *et al.*, 2012; Farfan *et al.*, 2015). In addition to complexity of the colonization and aflatoxin production have strong host and pathogen interactions (Amaike and Keller, 2011; Christensen and Kolomiets, 2011; Kelley *et al.*, 2012).

Keeping in view the importance of maize, current study was designed to assess the resistance of different maize genotypes to *A. flavus* and its influence on yield characteristics of field grown maize. In this study maize genotype(s), which were the superior in term of yield with resistance against *A. flavus* contamination were assessed to investigate the effects of *A. flavus* inoculation on grain yield of maize genotypes in open field environments at pre-harvest time, in Pakistan.

Materials and methods

Field experiment was set up under semi-arid climate of Pakistan in 2017, in the field of Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. This study involved total fourteen maize genotypes Pearl, MMRI,HC-9091, HC-2040,YH-1898, R-2315, R-33334, R-3305, R-2207, K.S.C 9663, K.S.C 9618, FH-949, FH-1046and Malka-16from Pakistan. Experiment was carried out in a randomized complete block design with five replications. Each plot had size of 5.0 x 0.75 m and inter row distance was kept 20 cm while inter plant distance was 75 cm. All cultural and agronomic practices were carried out as farmer used routinely.

A. flavus were cultured on the potato dextrose agar plate for 5-7 days at 28°Cand identified morphologically (Klich, 2002). The culture was prepared in broth potato dextrose and 1×10^4conidial concentration was made, that was enough for producing aflatoxin contamination in maize grains (Windham and Williams, 2016).

The fungal inoculums were applied to the cob through silk at grain filling stage. After applying the treatment of *A. flavus,* the rows were divided into two parts; non-inoculated and inoculated with *A. flavus*. After maturation of kernels, cobs were harvested by hand and data was recorded.

Meteorological data

The meteorological data, including temperature, rainfall and relative humidity of growing time from August to December, was obtained from the climatic station (about 300m from field) of the Department of Climate Change, Ayub Agriculture Research Institute Faisalabad (Pakistan). This data is presented in the Table 1.

Yield and yield-related parameters

Cob weight

Cob weight (g) was measured using a digital weighing balance (Ashraf *et al.*, 2016).

Cob length

To measure this physical trait, a total of 5 plants were selected and their lengths (cm) were measured using a measuring tape, and then an average value was recorded (Ashraf *et al.*, 2016).

Cob diameter

Cob diameter (cm) was measured by vernier caliper (Ashraf *et al.*, 2016).

Number of grains per Cob

Number of grains per cob was counted manually (Ashraf *et al.*, 2016).

Total Grain Weight per Cob

Total grain weight per cob (g) was measured in grams using a digital weighing balance (Ashraf *et al.*, 2016).

100 Grains Weight

Cob weight (g) was measured using digital weighing balance (Ashraf *et al.*, 2016).

Statistical analysis

The data was statistically analyzed by analysis of variance using Minitab-17 software and correlation by using SPSS version 24. The significance was determined at 0.05 probability level for each treatment.

Results

Meteorological data

Table 1 shows that the climatic conditions *viz.*, air temperature (10–35 °C), relative humidity (46–87 %) and rain fall (1.1–3.8 mm) recorded in the current study were favorable for fungal growth of *A. flavus* inoculation.

Yield related parameters

Cob length

Maize genotype, R-3305 produced highest cob length while maize genotype R-2315 and HC-2040 produced the lowest cob length (Fig. 1).

The *A. flavus* inoculated and non-inoculated plants showed a differential response with respect to cob length. Analysis of variance showed that for cob length there were highly significant differences among genotypes ($P < 0.001$) and significantly differences for treatments ($P < 0.05$), whereas Genotypes x Treatments interactions showed non-significant differences ($P > 0.05$; Table 2).

Cob diameter

Maize genotype FH-949 produced the highest cob diameter followed by the maize genotype MMRI and Pearl (Fig. 2). The *A. flavus* inoculated and non-inoculated plants showed a differential response with respect to cob diameter, which showed that cob diameter, was also not affected by the inoculation.

Table 1. Monthly average temperature, relative humidity and rain fall during the growing season of maize crop.

Months	Air Temperature (°C)		Relative Humidity (%)		Rain fall (mm)
	Max	Min	8:00 am	5:00 pm	
August	37.4	26.9	73.1	54.9	65.7
September	36.9	23.4	69.9	51.2	19
October	35.1	18.6	71.8	46.4	-
November	24.4	10.9	87.4	63.3	1.1
December	23.4	5.4	84.1	50	3.8

Analysis of variance showed that there were highly significant differences among genotypes and Genotypes x Treatments interactions ($P < 0.001$) and significantly different ($P < 0.01$) for treatments for cob diameter (Table 2).

Cob weight

Highest cob weight was observed in FH-949, followed by FH-1046, while maize genotype HC-2040 followed by HC-9091 and KSC-9618 produced the lowest value for cob weight (Fig. 3). Results showed that inoculum had reduced the cob weight.

Analysis of variance showed that cob weight had highly significant difference for genotypes and Genotypes x Treatments interactions ($P < 0.001$) and significantly different ($P < 0.01$) for treatments (Table 2).

Table 2. Analysis of variance for yield related characteristics of fourteen different maize genotypes under treatment of *A. flavus*.

	Df	Cob L	Cob D	Cob W	No. of grains /cob	Total grains W/ cob	100 grains W
Genotypes	13	6.68***	1.75***	15859***	78929***	6102.7***	327.98***
Treatments	1	0.16	0.63**	2979**	257572***	10393.6***	860.12***
Genotypes* Treatments	13	0.77*	0.84***	2900.8***	18935***	482.2*	62.28***
Error	112	0.78	0.11	362.8	5865	248.1	0.95
Total	139						

L=Length; D= Diameter; W= Weight;

(*, * *, *** Means square significant at $P < 0.05$, $P<0.01$ and $P < 0.001$, respectively.).

Number of grains per cob

Maize genotype R-3305 and MMRI produced the highest value for number of grains per cob followed by R-3334 and FH-1046 (Fig. 4). While the maize genotype, HC-2040 had lowest value for the number of grains per cob. Number of grains per cob was higher in non-inoculated plants as compared to the inoculated plants. Analysis of variance showed that there were highly significant differences among genotypes, treatments and Genotypes x Treatments interactions ($P < 0.001$) for number of grains per cob (Table 2).

Total grains weight per cob

Maize genotype FH-1046 produced the highest value for total grains weight per cob followed by FH-949 Pearl and YH-1898 (Fig. 5). Maize genotypes HC-2040 and HC-9091 produced the lowest value for the total grains weight per cob. Overall all inoculated plants showed decrease in total grain weight per cob as compared to the non-inoculated plants except HC-9091. Analysis of variance showed that there were highly significant differences among genotypes and treatments (P <0.001) whereas Genotypes x Treatments interactions had significant differences (P < 0.05) for total grains weight per cob (Table 2).

Table 3. Pearson correlation between maize genotypes, treatment of *A. flavus* and yield related traits.

	Treatments	Cob's Length	Cob's diameter	Cob's Weight	No. of Grains/Cob	Total grains weight/Cob	100 Grains Weight
Genotypes	0.87**	0.04	-0.08	-0.13	-0.33**	-0.295**	-0.29**
Treatments		0.030	0.116	-0.102	-0.34**	-0.29**	-0.38**
Cob's Length			0.099	0.22**	0.296**	0.32**	0.04
Cob's diameter				0.29**	0.11	0.26**	0.16
Cob's Weight					0.53**	0.75**	0.54**
No. of Grains/Cob						0.75**	0.21*
Total grains weight/Cob							0.57**

(Pearson correlation* and ** is significant at 0.05 and 0.01 probability level respectively).

100 grains weight

100 grains weight was affected by the inoculation and non-inoculated plants had higher values as compared with the non-inoculated plants (Fig. 6). Maize genotype KSC-9663 produced highest value for 100 grains weight followed by the FH-1046, Malka-16 and Pearl.

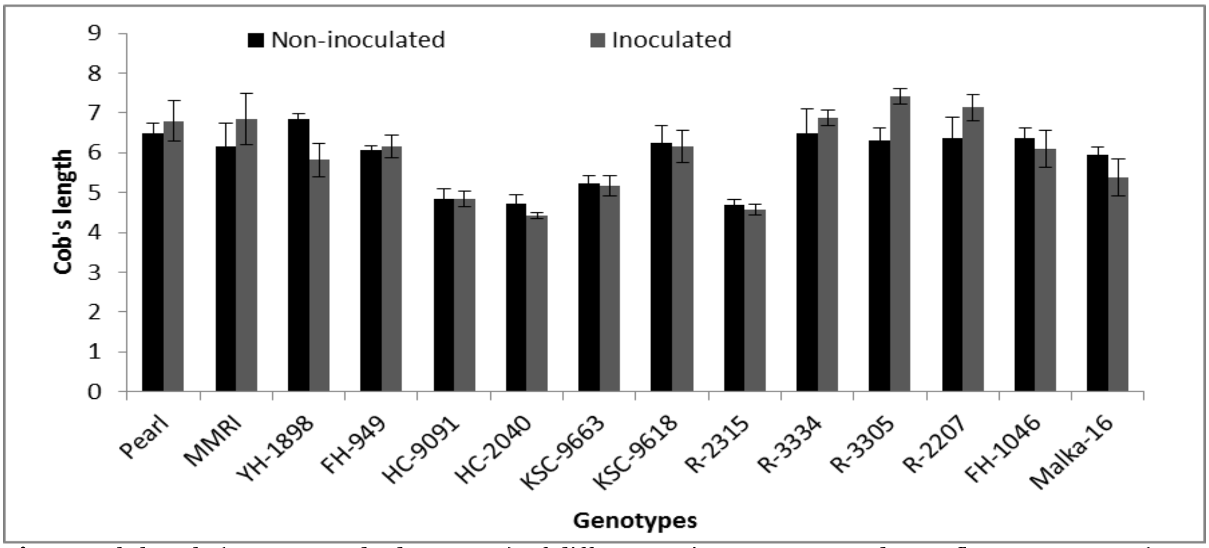

Fig. 1. Cob length (mean± standard error SE) of different maize genotypes under *A. flavus* treatment (Least Significant Difference at 0.05 probability level =1.12).

Analysis of variance showed 100 grains weight had highly significant difference for genotypes, treatments and Genotypes x Treatments interactions (P < 0.001; Table 2).

Correlation between genotypes, treatments yield related traits

Table 3 shows that there was a strong positive correlation between genotypes and treatments. Whereas, genotypes had strong negative correlation for number of grains per cob, total grains weight per cob and 100 grains weight. Treatments had strong negative correlation for number of grains per cob, total grains weight per cob and 100 grains weight.

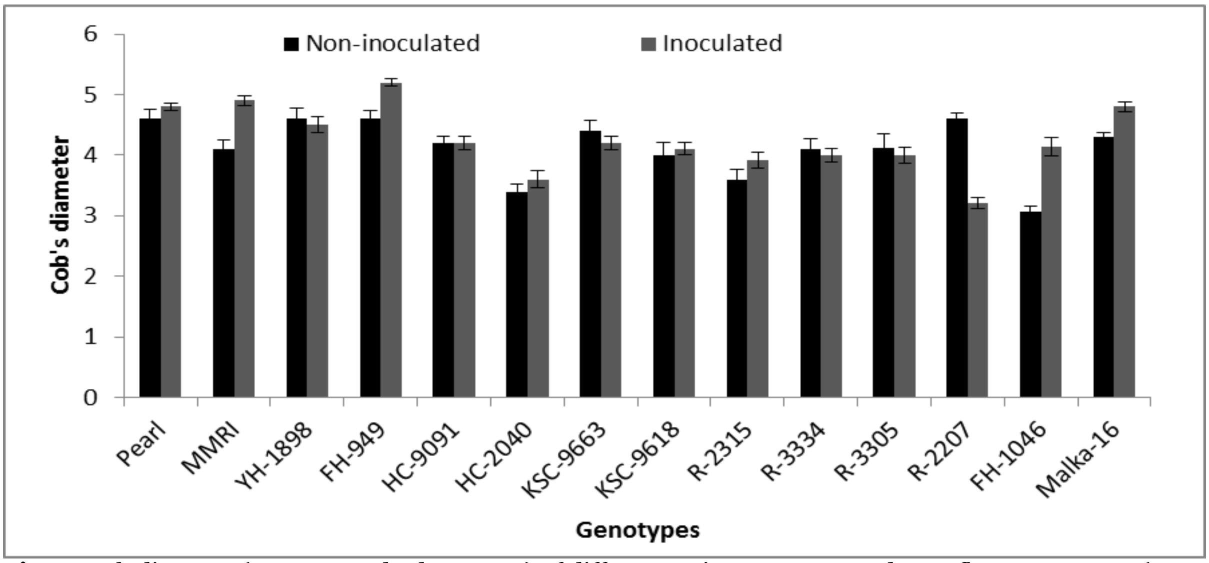

Fig. 2. Cob diameter (mean± standard error SE) of different maize genotypes under *A. flavus* treatment (Least Significant Difference at 0.05 probability level =0.42).

Cob length had strong positive correlation for cob weight, number of grains per cob and total grains weight per cob. Cob diameter had strong positive correlation for cob weight and total grains weight per cob. Cob weight had strong positive correlation for number of grains per cob, total grains weight per cob and 100 grains weight. Number of grains per cob had positive strong correlation total grains weight per cob and weak correlation for 100 grains weight. Total grains weight per cob had strong correlation for 100 grains weight.

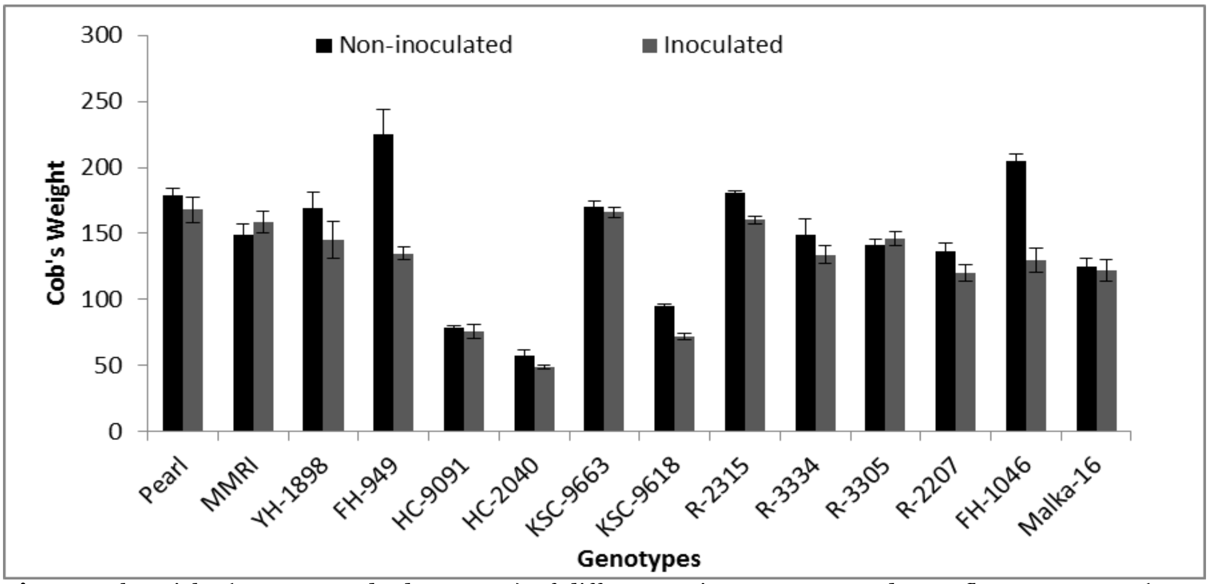

Fig. 3. Cob weight (mean± standard error SE) of different maize genotypes under *A. flavus* treatment(Least Significant Difference at 0.05 probability level =23.87).

Discussion

Meteorological parameters and A. flavus inoculation

Present study was conducted in a semi-arid conditions of Pakistan which is favorable for the growth of *A. flavus*. The present study revealed that inoculation had a negative impact on the maize yield. This is because; the concentration of the *A. flavus* inoculation was enough for producing the *A. flavus* infection and aflatoxin in the grains as earlier described by Windham and Williams (2016). In current study *A. flavus* treatment was applied through silk, which is very nearby to the natural infection process of *A. flavus* for production of aflatoxins. Windham and Williams, (2007) described that silk inoculation method may also be sufficient for *A. flavus* growth for production of aflatoxins, but it depend upon meteorological conditions, location and nature of maize germ plasm (Williams and Windham, 2012; Windham and Williams, 2016) and similar was observed in this study. Numerous previous studies described that *A. flavus* efficiently produced infection and ultimately aflatoxin under higher temperature (Bruns and Abbas, 2005; Campa *et al.*, 2005; Bruns and Abbas, 2006; Abbas *et al.*, 2007; Bellaloui *et al.*, 2016; Windham and Williams, 2016), similarly the current investigation had the temperature above 25°C.

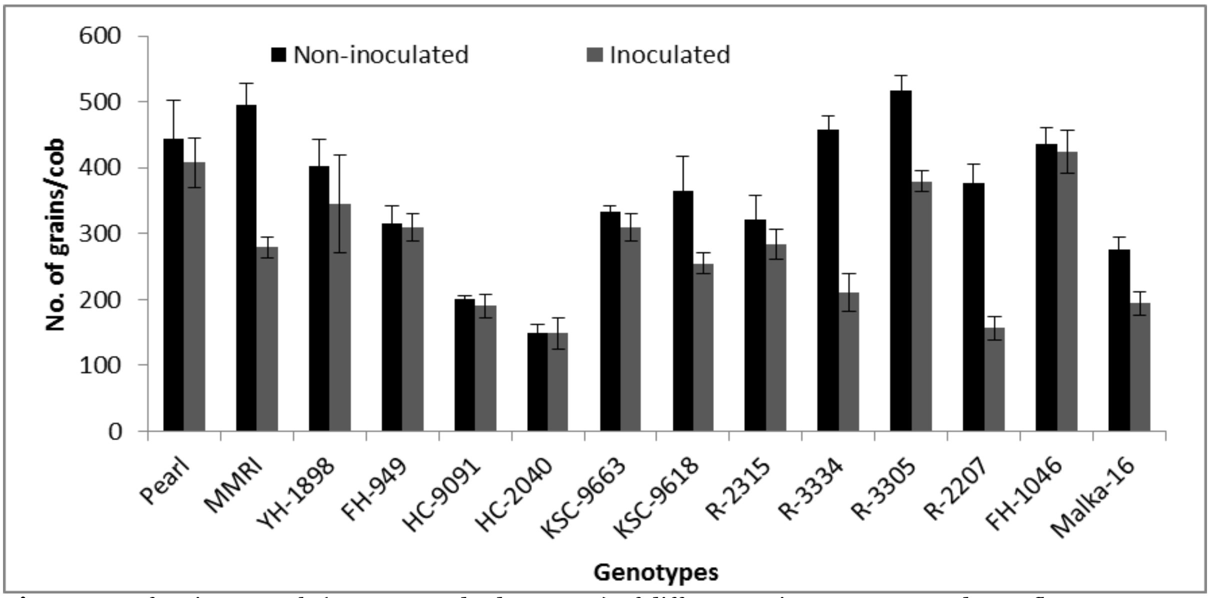

Fig. 4. No. of grains per cob (mean± standard error SE) of different maize genotypes under *A. flavus* treatment (Least Significant Difference at 0.05 probability level = 95.97).

Yield related parameters

The present study represented that *A. flavus* inoculated and non-inoculated plants showed a differential response with respect to cob length because the *A. flavus* treatment was applied during grain filling stage, when cob had achieved its length. Though, the maize genotype, HC-2040 showed relatively lower values than those of all other genotypes for almost all yield related parameters recorded during current investigation.

Maize genotype R-3305 had highest values for cob length and no. of grains per cob, whereas maize genotype FH-949 had higher values for cob diameter and cob weight as compared to all other genotypes investigated in the current study. Maize genotype FH-1046 produced higher value for total grains weight per cob and KSC-9663 produced higher values for 100 grains weight as compared to all other genotypes investigated in the current study. This differential behavior might be due to its genetic makeup as described in earlier studies(Grzesiak *et al.*, 2007; Ali *et al.*, 2011; Ali *et al.*, 2012a; Ali *et al.*, 2012b; Ali and Ahsan, 2015).

Present investigations revealed that treatments had strong negative correlation for number of grains per cob, total grains weight per cob and 100 grains weight. On the basis of such correlation, we can investigate the relationship between inoculation and yield.

The positive correlation of cob length with cob weight, number of grains per cob and total grains weight per cob was observed during present investigation such correlation was also observed by previous studies (Afarinesh *et al.*, 2005; Wang *et al.*, 2007; Ali *et al.*, 2013). Similarly the strong positive correlation was observed for cob diameter with cob weight and total grains weight per cob as well as cob weight had strong positive correlation for no. of grains per cob, total grains weight per cob and 100 grains weight. Such correlations were also observed by many scientists working on *A. flavus* and plants interaction (Afarinesh *et al.*, 2005; Grzesiak *et al.*, 2007; Ali *et al.*, 2011; Ali *et al.*, 2012a; Ali *et al.*, 2012b; Ali and Ahsan, 2015).

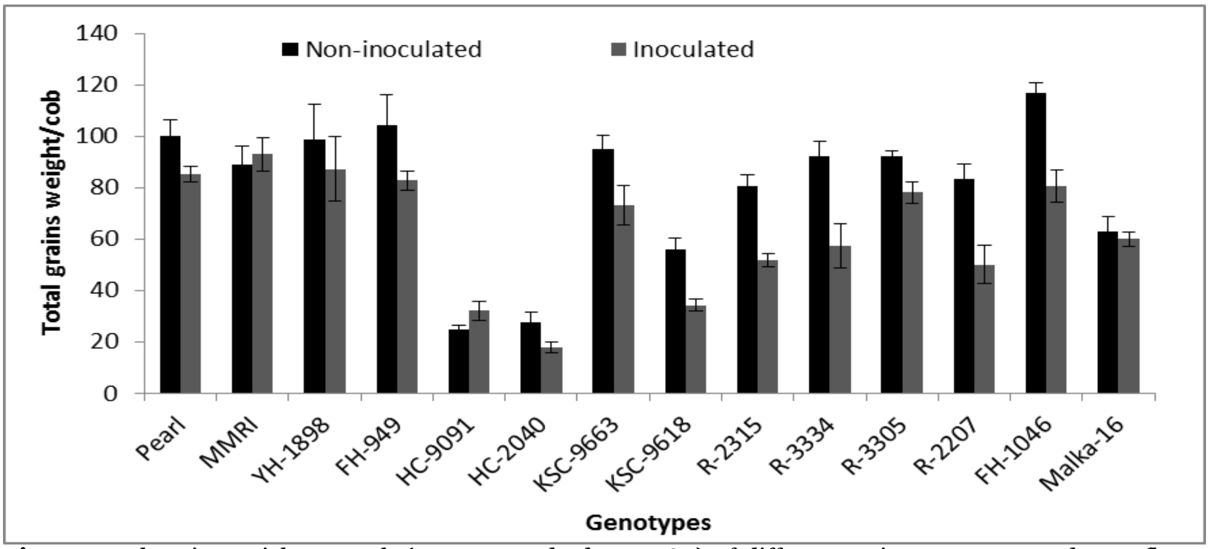

Fig. 5. Total grains weight per cob (mean± standard error SE) of different maize genotypes under *A. flavus* treatment (Least Significant Difference at 0.05 probability level = 19.74).

The findings of current investigations regarding positive correlations between yield and yield-related components of maize had also been reported by many other scientists (Ilker, 2011; Hasyan *et al.*, 2012; Kumar *et al.*, 2014; Karasu *et al.*, 2015). However, this could be considered as selection criteria for higher grain yield potential in maize genotypes (Rani *et al.*, 2017).

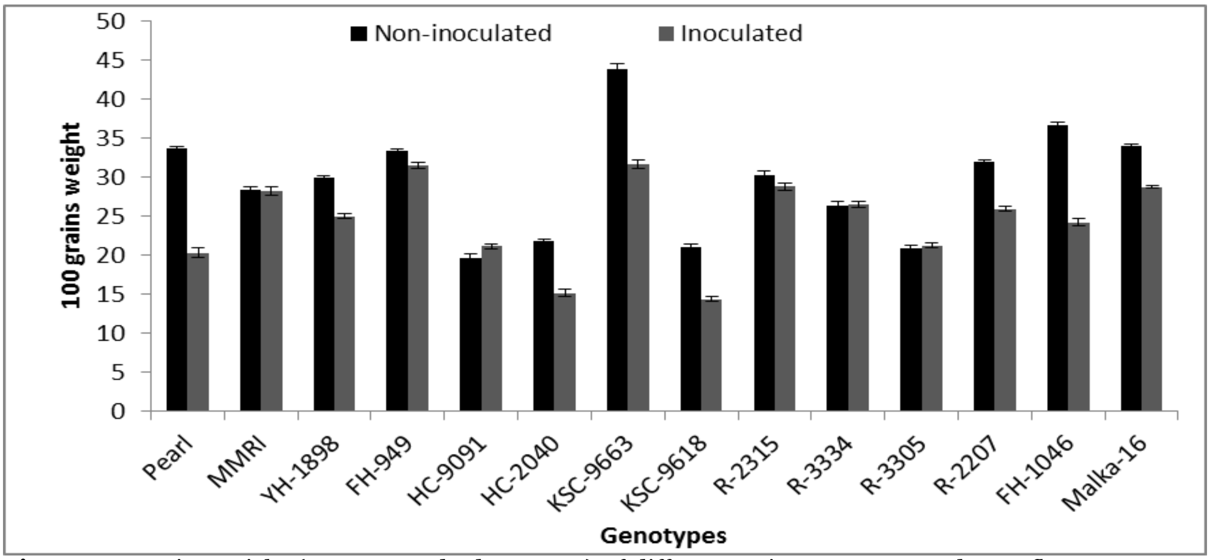

Fig. 6. 100 grains weight (mean± standard error SE) of different maize genotypes under *A. flavus* treatment (Least Significant Difference at 0.05 probability level =1.22).

Conclusion

It is concluded that *A. flauvs* treatment through silk could significantly affect the yield of maize genotypes under favorable weather conditions for *A. flauvs*.

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