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FIELD-BASED ASSESSMENT OF MAIZE (*ZEA MAYS* L.) GENOTYPES FOR RESISTANCE AGAINST *ASPERGILLUS FLAVUS* UNDER SEASONAL VARIABILITY

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SUMMARY

Maize (*Zea mays* L.) is the most important cereal crop worldwide and is highly susceptible to infection by *Aspergillus flavus*. This pathogenic fungus can cause ear rot and produce toxic aflatoxin contamination in maize kernels. This contamination poses serious economic and health risks globally. Limited knowledge of maize resistance mechanisms to this fungus presents a challenge for scientists and farmers trying to select resistant varieties. This field trial aimed to assess the maize responses to *A. flavus* exposure during the autumn and spring of 2023–2024. Maize cobs from both inoculated and non-inoculated plants were harvested and shade-dried before analysis for various parameters. Results showed non-inoculated maize genotypes performed better than those inoculated with *A. flavus*, despite minimal visible infection symptoms across all genotypes. Exposure to *A. flavus* negatively affects the contents of protein, fat, carbohydrates, fiber, and moisture of grains. The principal component analysis identified PBG-35, PBG-43, and PBG-16 as the top-performing genotypes, while PBG-80, PBG-81, and PBG-83 performed poorly under both conditions. The results revealed that inoculation with *A. flavus* reduces maize grain yield and quality even without visible infection signs. These resistant genotypes can be desirable in developing *Aspergillus* ear rot-resistant maize hybrids with a reduced risk of aflatoxin contamination through breeding programs.

Keywords: Maize (*Z. mays* L.), fungal infection, cob, inoculation, disease resistance, *Aspergillus flavus,* ear rot, seasonal variability, grain quality

Key findings: The changing climatic pattern worldwide causes significant biotic stress on maize (*Z. mays* L.), specifically *Aspergillus flavus* infection, ear rot, and aflatoxin contamination. Addressing these issues requires the identification and screening of resistant maize germplasm. Findings highlight the importance of disease-resistant germplasm for developing disease-resistant hybrids in maize breeding programs.

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INTRODUCTION

Maize (*Zea mays* L.) has the common name as the Queen of cereals, and it has a unique position in the grains and agriculture world. Maize is a major staple crop for billions of people worldwide, ranking as the second most produced grain in the world after wheat, and a significant grain that is not vital only for food security but also for industries (Gomes *et al.*, 2024; Swati *et al.*, 2024). In Pakistan, maize is the third most produced crop after wheat and rice. Maize cultivation occurs on 1.59 million hectares, yielding a total production of 8.79 million tons. It contributes about 2.3% of the total value added in agriculture and accounts for around 0.5% of the country's gross domestic product (GDP). GDP is the total monetary value of all finished goods and services produced within a country's borders during a specific period (GOP, 2025–2026). Maize is a major source of nutrients like proteins, lipids, vitamins, carbs, fibers, and minerals, providing approximately 15% dietary protein and 20% of daily calories (Amanjyoti *et al.*, 2024). Influences on its production include various biotic factors, which can significantly reduce yield and quality. An estimate reveals that microbial infestations and insect attacks lead to a 9.4% decline in total world maize production (Oppong *et al.*, 2022).

Disease-related maize yield losses have an estimate of 22.5%, with global losses extending between 19.5% and 41.1% (Savary *et al.*, 2019). Fungal pathogens are one of the numerous pathogenic agents of maize, which cause more than 110 diseases and massive losses in food production globally. The quality and yield of maize highly incur effects from these diseases. Nutritional value is low, and it produces mycotoxins that pose serious health risks to human and animal health and economic loss because of low grain quality and marketability (Shabana *et al.*, 2022). Maize crops are vulnerable to contamination from toxigenic fungi at various stages of production, including cultivation, harvesting, storage, transportation, and processing. About 25% of maize and its products acquire mycotoxin contamination (Zhang *et al.*, 2024). In maize, common fungi, such as *A. flavus*, *A. nomius*, and *A. parasiticus,* produce aflatoxins (AFs), especially after harvest. *A. flavus* attacks the ears in maize and leads to *Aspergillus* ear rot, diagnosed as a powdery yellow-green or olive-green mold on the kernels (Zakaria, 2024).

One of the concerns associated with *Aspergillus* ear rot is the production of aflatoxins, which are cancer-causing and toxic compounds that could emerge on the grain without any visible symptoms. Moisture, high temperatures, drought, and delaying harvest all contribute to *Aspergillus* infections and aflatoxin accumulation in crops. Estimates reveal that aflatoxin contamination affects over 4.5 billion people worldwide every year. With the health risks involved, the enactment of strict rules arose in most countries regarding the content of aflatoxins in food and feed (Shabeer *et al.*, 2022). Most farmers in Pakistan practice maize farming in subtropical areas and receive nearly 65% average annual rainfall in monsoon months between June and August.

The warm and humid environment of Pakistan supports the growth of *Aspergillus* species, which leads to the flavus infection and subsequent contamination of maize with aflatoxins. The *flavus* synthesizes numerous forms of aflatoxins, among which AFB1, AFG1, AFG2, and AFB2 are mostly measurable (Asghar *et al.*, 2020). In Pakistan, some studies report aflatoxin contamination levels in maize samples, ranging from 28% to 80%. Nevertheless, none of the control strategies is fully efficient in the case of an ideal environment of fungus proliferation. The most effective control method is the development of maize hybrids resistant to aflatoxin contamination (Asghar *et al.*, 2020; Barosevic *et al.*, 2022).

This research hypothesized that *A. flavus* infection differentially affects the yield and grain quality of maize genotypes across seasons, which allows the identification of resistant genotypes for breeding purposes. The current study aimed to determine the effect of *A. flavus* inoculation on yield and grain quality of maize genotypes under field conditions and compare seasonal (autumn and spring) variation in maize genotypes against *A. flavus* infection. Likewise, the study sought to identify

the *A. flavus*-resistant maize genotypes for breeding programs aimed at reducing aflatoxin contamination risks. The findings will be helpful for regulatory agencies that monitor and control the use and export of contaminated maize.

MATERIALS AND METHODS

The field trials on maize were conducted at the experimental site in the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan (latitude 31°44′ N, longitude 73°06′ E, elevation 184.4 meters above sea level). The field trials were carried out in autumn 2023 and spring 2024; the research sought to identify the maize genotypes resistant to *A. flavus* with improved grain nutritional quality and yield.

Collection of germplasm

Fifty maize genotypes were collected from the following institutions: Maize and Millet Research Institute (MMRI), Sahiwal; Plant Genetic Resource Institute (PGRI), Islamabad; Cereal Crops Research Institute (CCRI), Nowshera; International Maize and Wheat Improvement Center (CIYMMT); and Maize Breeding and Genetic Resource Lab at the Department of Plant Breeding and Genetics (PBG), University of Agriculture, Faisalabad (UAF), Pakistan.

Experimental design

The experiments, carried out in the autumn of 2023 and spring of 2024, used a randomized complete block design (two-factor) with three replications. The study maintained a plant-to-plant and row-to-row spacing of 25 cm and 75 cm. Standard agronomic practices include hoeing, weeding, irrigation, pest control, and fertilizer application (92 kg N, 58 kg P_2O_5, 37 kg K_2O per 0.404 ha (or per acre), as per the Punjab Maize Production Plan).

Experimental time and meteorological conditions

Meteorological data, including relative humidity, rainfall, and temperature, were collected from the Meteorological Station, University of Agriculture, Faisalabad, Pakistan (Table 1).

Preparation and application of fungal inoculum

The most native fungal isolate of *A. flavus* with the highest ear rot and subsequent aflatoxin production ability acquired from the Maize and

Table 1. Monthly pattern of relative humidity, rainfall, and temperature during the experiments of the maize crop in the autumn of 2023 and the spring of 2024.

During the autumn of 2023							
Months	Relative Humidity (%)			Rainfall	Temperature (°C)		
	Maximum	Minimum	Average	(mm)	Maximum	Minimum	Average
August	83	60	66.3	12.3	45	23.5	34.2
September	89	58	68.5	95	41	20	31.6
October	88	60	73.4	30.7	38	15	25.4
November	77	57	68.6	3.8	32	9	20.5
December	91	62	70.1	0	26	6	15.1
During the spring of 2024							
Months	Relative Humidity (%)			Rainfall	Temperature (°C)		
	Maximum	Minimum	Average	(mm)	Maximum	Minimum	Average
February	70	44	58.1 %	7.5	27	5.0	16
March	84	42	55.5	5.8	35	5.0	19.8
April	85	38	49.5	21.2	39	11	25.8
May	58	20	36.5	15.6	48	16	33.3
June	58	29	41	19.6	48	19	35.2

Millet Research Institute, Sahiwal. This underwent sub-culturing on a potato dextrose agar (PDA) in a biosafety cabinet (BSC-1100IIA2-X), following the procedure of Khan *et al*. (2020). A seven-day-old fungal culture preceded inoculation, with the broth continuously shaken at 150 rpm and room temperature for seven days until a dense, yellowish-green growth of *Aspergillus* appeared. The concentration of conidia was quantified using a hemocytometer and adjusted to achieve a final density of 6×10^7 conidia per mL, while a 5 mL conidial suspension was used for inoculation by the side-needle inoculation method (Oppong *et al.*, 2022).

Data collection

Hand-harvesting of maize cobs occurred at kernel maturity. The attributes related to grain quality, including protein (P), fiber (F), fat (F), ash (A), and moisture (M), sustained measurement using a Near-Infrared Spectroscopy Analyzer (Simeone *et al.*, 2024). Meanwhile, the calculation of total carbohydrate content was determined by difference using the formula below (Oluwadiran *et al*., 2025).

$$\text{Total carbohydrate percentage} = 100 - (A+F+P+M+F)$$

The internodal length, days to tasseling and silking, anthesis-silking interval, and leaf area were the recorded traits. Grain yield recording continued by taking the mean of the weights of threshed bulked 10 samples on an electric balance. At physiological maturity, the harvested ears were rated for the severity of ear rots using a modified 1–6 scoring scale (Table 2). The collected data of grain samples were analyzed by using the ANOVA in Minitab, and performing correlation, heat map, and PCA used R software at a significance level of $p \leq 0.05$.

RESULTS

Analysis of variance

The difference between the inoculated and non-inoculated groups of maize, when tested, used the analysis of variance. The ANOVA applied to the recorded data was on 50 inbred lines at 5% significance, revealing the high variability in all the experimentally recorded traits across genotypes and treatments. Table 3 illustrates the mean square values of examined traits during non-inoculated and inoculated groups in the autumn and spring of 2023 and 2024, respectively, which provided information regarding data trends. In this study, Table 1 presenting the recorded data indicates that relative humidity levels (20%–85%), rainfall (7.5–19.6 mm), and air temperatures (5 °C–48 °C) were most suitable for the growth of *A. flavus* inoculation under field conditions.

Correlation analysis

Using a correlogram helped to represent the association graphically, as given in Figures 1a and 1c under non-inoculated conditions (autumn and spring of 2023 and 2024, respectively). Leaf area has a positive correlation with grain yield per plant, and internode length is in a negative correlation with grain yield per plant. The anthesis-to-silking interval also represents a negative

Table 2. The modified disease rating scale used for disease estimation and host reactions.

No.	Disease rating (%)	Host reaction	Reported by
1	0-infection	Highly Resistant	Reid *et al.*, 1996; Oppong *et al.*, 2022
2	1-25	Resistant	
3	26-50	Moderately Resistant	
4	51-75	Moderately Susceptible	
5	76-99	Susceptible	
6	100	Highly Susceptible	

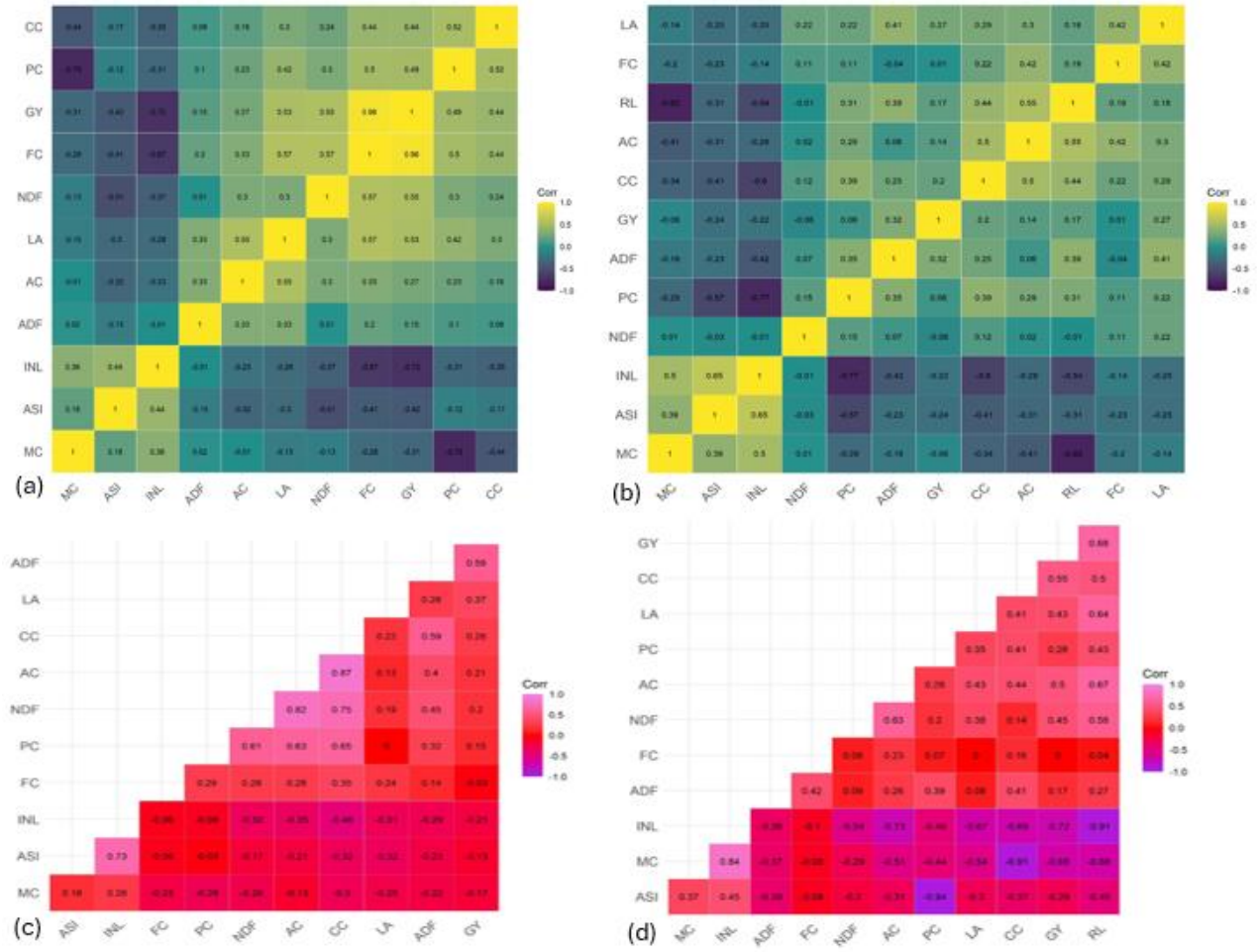

Figure 1. Represents Correlogram which shows the interrelationship among different parameters of studied genotypes in inoculated (Figures 1b and 1d) and non-inoculated (1a and 1c) conditions of the autumn of 2023 (a and b) and spring of 2024 (c and d). (MC: Moisture content, ASI: Anthesis to silking intervals, INL: Internode length, ADF: Acidic detergent fiber, AC: Ash content, LA: Leaf area, NDF: Neutral detergent fiber, FC: Fat content, GY: Grain yield per plant, PC: Protein content, CC: Carbohydrate content, and RL: Resistance level).

correlation with grain yield per plant. Under inoculated conditions of both seasons (Figures 1b and d), the moisture content that favors the growth and proliferation of *A. flavus* showed a strong negative correlation with resistance levels. Meanwhile, the trait of resistance levels was in a positive association with quality traits, such as carbohydrate, protein, fat, and fiber content.

Heat map analysis

The heat map of autumn 2023 under non-inoculated conditions appears in Figure 2a. Regarding moisture content, PBG-85 showed the highest, while PBG-83 had the lowest mean values. The high anthesis-to-silking interval and internode length resulted in PBG-35. For acidic detergent fiber, PBG-4 demonstrated the

highest mean value, while PBG-120 provided the lowest. For ash content, PBG-41 had the maximum, while PBG-17 had the minimum mean value. The highest leaf area was evident in PBG-84, while PBG-35 expressed the lowest. Concerning the neutral detergent fiber content, PBG-90 and PBG-81 displayed the highest means, while PBG-35 showed the lowest. For fat content and grain yield per plant, PBG-81 and PBG-80 had the maximum mean values, while PBG-35 and PBG-120 had the minimum. The protein content emerged to be highest in PBG-83, while PBG-29 had the lowest mean value. For carbohydrate content, PBG-38 disclosed the maximum, while PBG-22 had the lowest mean value.

The heat map data of the inoculated conditions in autumn of 2023 is available in Figure 2b. An inverse relationship between the protein content and the anthesis-to-silking interval existed among the two genotypes; PBG-63 had the shortest interval, shortest internode length, and the highest protein content and grain yield per plant, whereas PBG-16 had the longest interval, the longest internode length, and the lowest protein content and grain yield per plant. The maximum neutral detergent fiber, protein, and leaf area occurred in PBG-83, and it consistently recorded high values. Overall, the performance of PBG-43 was poor, having the highest moisture but the lowest carbohydrate content, resistance, and leaf area. High acid detergent fiber, fat content, leaf area, and low grain yield were outcomes from PBG-48. The low-acid detergent fiber and high grain yield resulted from PBG-17, which also had high resistance. PBG-8 contained the highest amount of ash content, and PBG-76 contained the least amount of ash content.

The heat map of the studied traits under non-inoculated conditions in the spring of 2024 is available in Figure 2c. PBG-83 exhibited the lowest moisture, anthesis-to-silking interval, and internode length and the highest protein content, while all these traits showed the opposite mean values for PBG-95. PBG-76 showed the lowest acid detergent fiber, carbohydrate, and ash content, even though it had the highest acid detergent fiber. The leaf area and acid detergent fiber were low in PBG-17, and moisture was low in PBG-36, with the lowest grain yield per plant. The highest grain yield obtained was with PBG-80, while the highest neutral detergent fiber (NDF) came from PBG-90.

The heat map of inoculated conditions of spring 2024 data appear in Figure 2d. PBG-14 was the best performer in all aspects of the study, with low moisture and internode length and the highest ash content, leaf area, carbohydrate content, grain yield, and resistance. Likewise, PBG-83 had the highest ash content, leaf area, carbohydrate content, grain yield, and resistance, with low moisture and internode length. Compared with PBG-121, both differed in their moisture and internode length but not in the neutral detergent fiber, ash, leaf area, and resistance. For example, PBG-4 exhibited a low anthesis-to-silking interval, high acid detergent fiber, fat, and resistance, whereas PBG-95 displayed a high anthesis-to-silking interval with low acid detergent fiber and protein. The PBG-8 was unique because it had high levels of fat, fiber, and protein.

Principal component analysis

In this study, data of both seasons were analyzed using four independent principal component analyses (PCA) (Figures 3a-d). The first for the control group of the autumn of 2023 (non-inoculated) (Figure 3a) accounted for 57% of the total variability explained by the first two principal components (PC1 = 42.3% and PC2 = 14.7%). The second PCA in the autumn of 2023, as performed for the traits of genotypes inoculated with *A. flavus* (Figure 3b), it explained 47.8% of the total variability, with the first two principal components accounting for PC1 = 36.1% and PC2 = 11.7%. The third PCA for the control group of the spring of 2024 (non-inoculated) (Figure 3c) accounted for 55% of the total variability explained by the first two principal components (PC1 = 39.3% and PC2 = 15.7%). The fourth PCA for the spring of 2024, performed for the traits of genotypes inoculated with *A. flavus* (Figure 3d), this explained 62.4% of the total

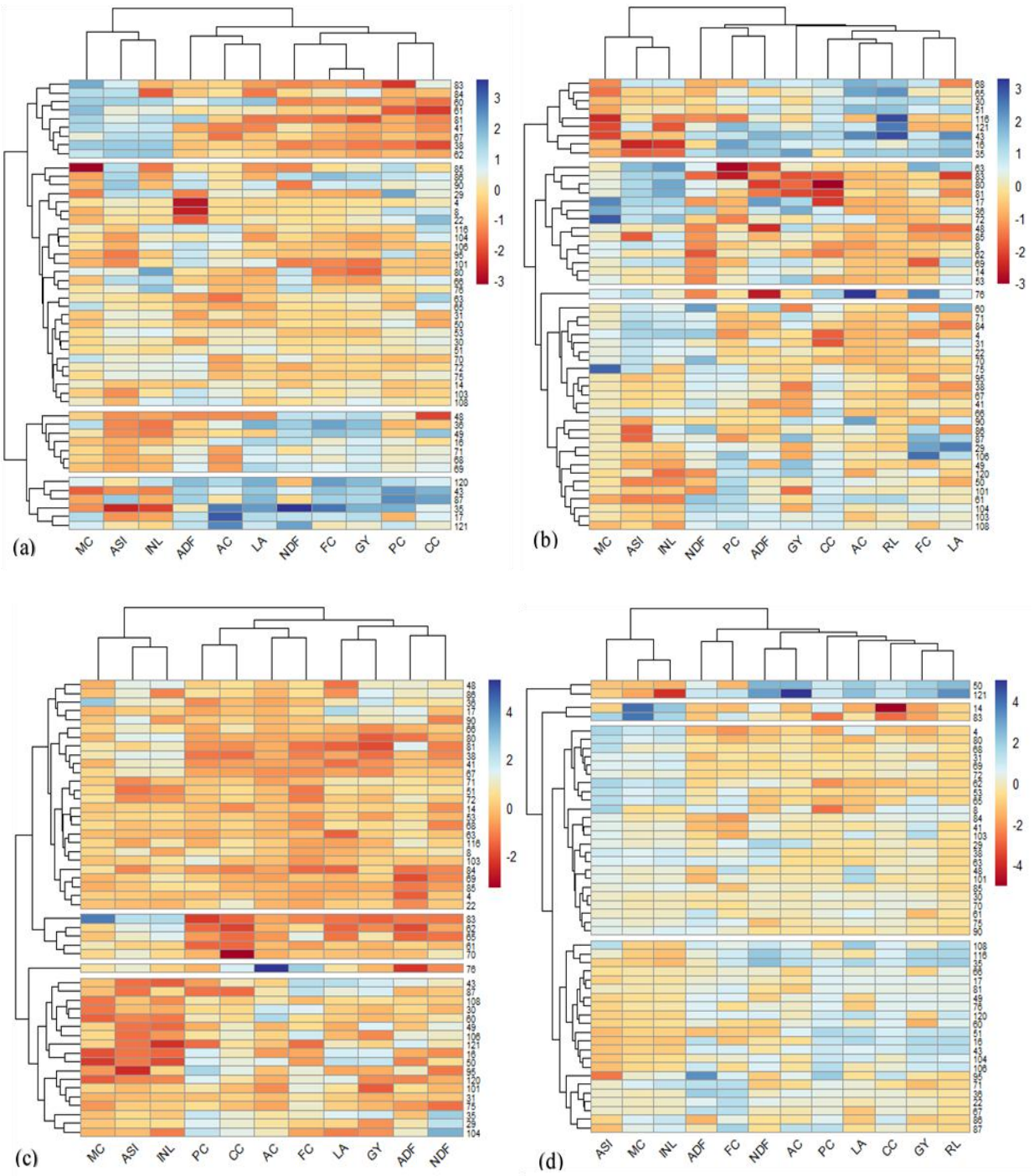

Figure 2. Represents a Heat Map of the inoculated (Figures 1b and 1d) and non-inoculated (1a and 1c) conditions of the autumn of 2023 (a and b) and spring of 2024 (c and d) for various yield and quality-related traits in maize genotypes, which indicate the relationship among genotypes and studied parameters. (MC: Moisture content, ASI: Anthesis to silking intervals, INL: Internode length, ADF: Acidic detergent fiber, AC: Ash content, LA: Leaf area, NDF: Neutral detergent fiber, FC: Fat content, GY: Grain yield per plant, PC: Protein content, CC: Carbohydrate content, and RL: Resistance level).

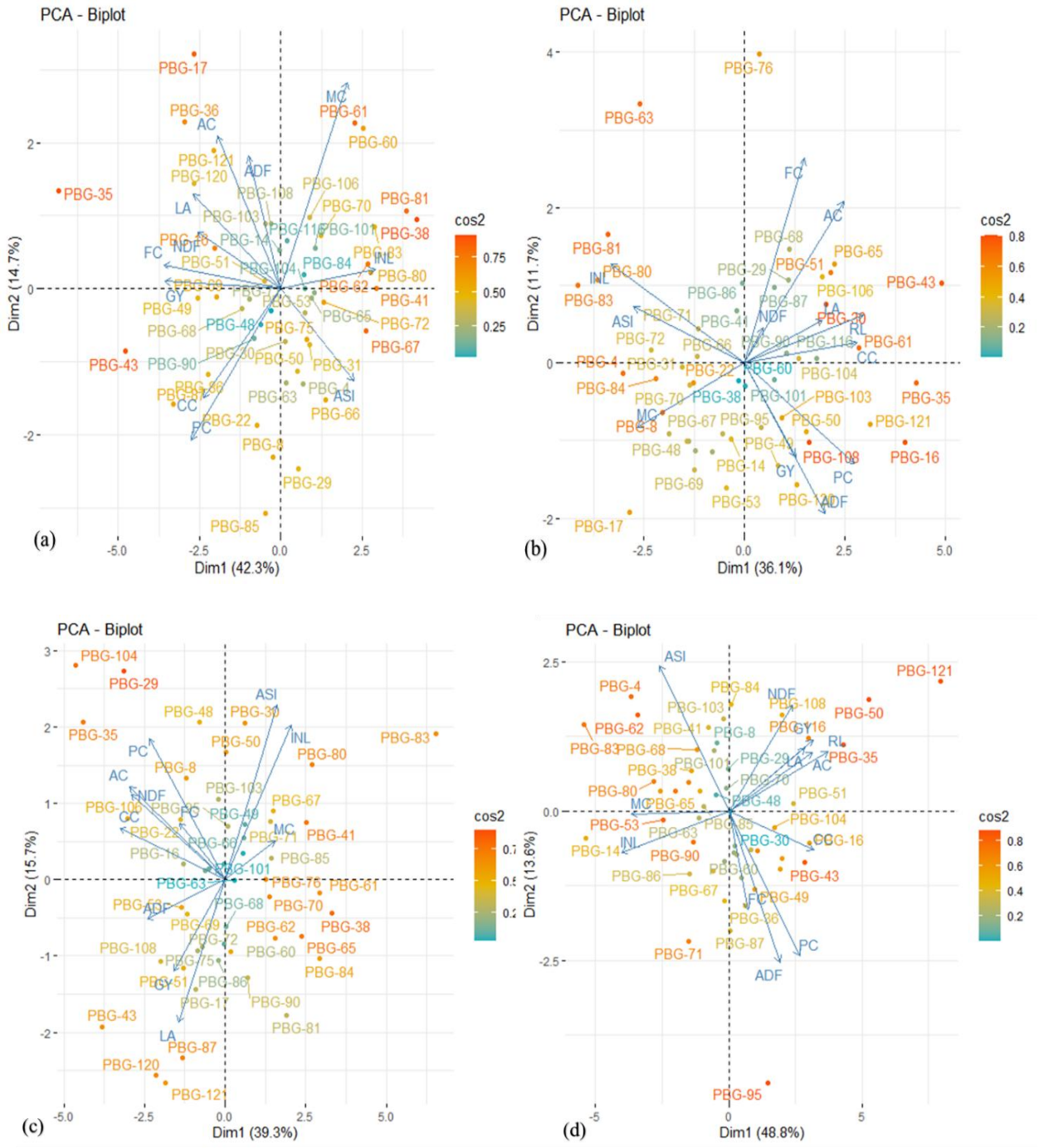

Figure 3. Represents the Principal Component biplot of the inoculated (Figures 1b and 1d) and non-inoculated (1a and 1c) conditions of the autumn of 2023 (a and b) and spring of 2024 (c and d) for the studied genotypes. The biplot illustrates the distribution of genotypes across the studied parameters, facilitating the identification of the best genotype-parameter combinations. (MC: Moisture content, ASI: Anthesis to silking intervals, INL: Internode length, ADF: Acidic detergent fiber, AC: Ash content, LA: Leaf area, NDF: Neutral detergent fiber, FC: Fat content, GY: Grain yield per plant, PC: Protein content, CC: Carbohydrate content, and RL: Resistance level).

Table 3. The analysis of variance of data representing agronomic, quality, and yield-related parameters of studied maize genotypes under inoculated and non-inoculated conditions during the autumn of 2023 and spring of 2024.

Autumn 2023													
SOV	Df	Moisture content (%)	Protein content (%)	NDF (%)	ADF (%)	Ash content (%)	Fat content (%)	Carbohydrate content (%)	ASI (days)	Leaf area (mm)	Internode length (mm)	Grain yield per plant (g)	Resistance levels (%)
Replications	2	5.59	4.64	1.32	2.80	3.99	0.49	33.386	4.61	179034	10.71	382.68	0.2
Genotypes	49	6.89 ***	6.25***	2.37***	14.85**	49.62***	0.39***	58.874***	1.70*	71531***	12.56***	1556.89***	1028.5 ***
Treatments	1	20.18***	70.08***	10.45*	114.58*	1014.94**	15.14***	523.77***	1.33ns	25098ns	10.30ns	3047.09*	32085.0***
Genotypes* Treatments	49	7.10***	4.00***	1.77*	15.39**	53.80***	0.48***	49.705***	0.61ns	4821ns	1.47ns	167.79**	1028.5***
Error	198	0.32	1.51	0.09	0.19	0.11	0.08	1.9	1.02	7644	2.99	48.23	0.10
Total	299												
Spring 2024													
Replications	2	16.65	2.81	0.64	3.24	1.64	0.05	50.60	0.46	285.50	0.17	92.91	3.70
Genotypes	49	6.94***	7.15***	1.82***	16.22***	13.80***	0.54***	18.10***	3.10***	97156.80***	16.85***	103.61***	702.90***
Treatments	1	107.40**	103.49***	36.96*	1846.1***	3.61**	16.10**	32.74***	0.48ns	1417.00ns	0.09ns	1899.89***	24764.8***
Genotypes* Treatments	49	2.81***	3.70***	1.16***	12.94***	14.65**	0.49**	19.59***	0.51ns	408.20ns	0.50ns	7.68***	702.90***
Error	198	0.10	0.17	0.05	0.09	0.06	0.07	0.41	0.46	3371.60	1.01	2.80	0.80
Total	299												

Ns = Non-significant * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, and df = degree of freedom.

variability, with the first two principal components accounting for PC1 = 48.8% and PC2 = 13.6%. The best-performing genotypes in both seasons, including PBG-35, PBG-43, and PBG-16, obtained selection based on their trait vector on the PCA biplot, while the poor-performing genotypes were identifiably PBG-80, PBG-81, and PBG-83.

DISCUSSION

Crop development initiatives for disease resistance rely primarily on genetic diversity and parental selection, which are crucial for enhancing crop resilience to various stresses (Campbell *et al*., 2025; Pati *et al*., 2025). The assessment of 50 maize inbred lines under non-inoculated and inoculated conditions during the autumn of 2023 and the spring of 2024 revealed considerable variations across traits, indicating potential utility and heritability (Table 3). The presented study, conducted under semi-arid conditions of Pakistan, is conducive to the growth of *A. flavus*. The results demonstrated that inoculation with *A. flavus* adversely affected maize yield, quality, and yield-related traits. The findings of both screening experiments (Autumn 2023, Spring 2024) clearly revealed that several traits, including grain yield per plant, grain carbohydrate percentage, protein, fat, fiber, and moisture content, were lower in inoculated plants than in non-inoculated ones. Similar results were in other studies, including reductions in quality and yield traits (Dolezal *et al.,* 2014; Muga *et al.,* 2019; Oppong *et al*., 2022).

After infection of maize, *A. flavus* secreted several metabolic hydrolytic enzymes, particularly proteases, lipases, and amylases, which degraded starch, proteins, and lipids (Dolezal *et al.,* 2014). The climatic data in Table 1 revealed that relative humidity, temperature, and rainfall were within the range required by *A. flavus* for growth and infection. The most commonly reported

growth temperatures for *A. flavus* were a minimum of 10 °C–12.8 °C and a high of 43 °C–48.8 °C, with 30 °C–33.8 °C being the optimal temperature for *A. flavus* infections. In another study, however, *Aspergillus* grows from 19 °C to 35 °C, while 28 °C is optimum for growth, and 28 °C–30°C is superior for the production of aflatoxin (AL-Warshan and AL-Hadethy, 2012; Casu *et al.,* 2024). A research study has hypothesized that low amounts of inoculum may not be efficient in producing visible fungal infection. The concentration of inoculum selected as the most effective in the latest study was sufficient to induce *A. flavus* infection, *Aspergillus* ear rot, and aflatoxin contamination in maize grains. Another possible reason for the lack of clear visible symptoms of fungal infection may be the use of resistant genotypes (Windham and Williams, 2016; Barosevic *et al*., 2022).

Germplasm assessment using the side-needle inoculation technique is a successful and potentially more accurate method of causing infections. Other researchers stated that the chosen method is similar to the natural infection of maize with *A. flavus*, with its efficiency also determined by site, weather, and the traits of the maize germplasm. The presented research revealed the inoculation by *A. flavus* affected different parameters of the study despite the presence of climatic conditions favorable to the growth of *A. flavus*. This effect is perhaps due to the time of inoculum application at the mid-silking stage, when the cob development is still in progress (Windham and Williams, 2016). Moreover, another study found the side-needle technique yielded a higher fungal load and higher levels of aflatoxin accumulation than with non-wounding silk channel inoculation, further supporting the increased sensitivity to detect host-pathogen interactions (Wahl *et al.*, 2019). In this current research, this technique has been able to detect losses in quality and yield due to *A. flavus,* even in the absence of ear rot symptoms. It reveals the same results (Mideros *et al.*, 2009) that maize grain could acquire contamination from aflatoxin without visible infection signs.

The comparatively high grain yield values of some genotypes suggest that they possess high production potential compared with other genotypes tested in the current study. The small reduction in the grain yield of resistant genotypes following *A. flavus* treatment and the positive correlation between the abovementioned traits revealed the resistance to the pathogen and the yield potential of the resistant genotypes. The quality parameters, recorded in the latest research, are consistent with values reported in previous maize studies (Simeone *et al.*, 2024).

Throughout the study, leaf area gave a positive correlation with grain yield per plant, and internode length is in a negative correlation with grain yield per plant, as reported earlier (Bernhard and Below, 2020). The anthesis-to-silking interval also represents a negative correlation with grain yield per plant (Lima *et al*., 2023). Under-inoculated conditions of both seasons (Figures 1b and d), the grain moisture content that favors the growth and proliferation of *A. flavus* showed a strong negative correlation with resistance levels. Meanwhile, the trait of resistance levels indicated a positive association with yield and quality, as reported in other studies. In another study, 16 genes identified via genome-wide association study (GWAS) in maize kernel signified involvement in resistance to *A. flavus* infection (Mesterhazy *et al*., 2024). The development of *A. flavus* and the later production of aflatoxin mostly rely on the degree of carbohydrate content in grains, as supported by reports from various studies. This difference in genetic composition might be the reason behind variations in genotypic performance, as reported by various research studies; it is best articulated when relating to a study involving inoculations with *A. flavus* (Barosevic *et al.*, 2022; Mohammadi *et al.*, 2022). On a practical level, each of the three top-performing screen genotypes (PBG-35, PBG-43, and PBG-16) would offer the breeder readily available parental lines to be used for the production of *Aspergillus* ear-rot-resistant hybrids.

CONCLUSIONS

Genetic diversity plays a vital role in the identification of crop varieties resistant to different diseases. This experiment inoculated 50 maize genotypes with *A. flavus* for the identification of agronomic, quality, disease-resistance, and yield-related traits. *Aspergillus flavus* inoculation adversely affected the quality and yield of maize genotypes even in the absence of detectable symptoms. *A. flavus* infection, ear rot, and aflatoxin contamination required combined strategies for safe crop production and human health. Genetic and phenotypic responses to fungal inoculation are important for breeding resistant crop varieties, ensuring long-term agricultural productivity, and food safety. This research provides valuable germplasm screening information to support breeders in developing resistant hybrids, as well as helping farmers to reduce fungal infection and aflatoxin contamination risks by growing resistant hybrids.

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