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Corn (*Zea Mays* L.) resistance to mycotoxin-producing fungi: Genetic and agronomic approaches to reduce contamination

Dupe Stella Ogundipe *

Department of Agricultural Science, Florida A & M University, FL., USA.

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Abstract

This study investigates the genetic, physiological, and biochemical traits associated with drought tolerance and mycotoxin resistance in five corn cultivars: Pioneer 30F35, Dekalb DKC63-55, Golden Harvest G12W66, AgriGold A6533, and Channel 214-00. SNP markers linked to water-use efficiency (WUE) and proline accumulation were found in Pioneer 30F35 and Channel 214-00, suggesting a genetic basis for drought resilience. Biocontrol application reduced aflatoxin levels by 55% in Channel 214-00 and AgriGold A6533, demonstrating effective mycotoxin management. Physiological responses, including proline and soluble sugar accumulation, contributed to osmotic adjustment, while enhanced antioxidant enzyme activity mitigated oxidative stress in Pioneer 30F35 and Dekalb DKC63-55. Root architecture and hydraulic conductance supported water uptake, and crop rotation with non-host plants significantly decreased soil fungal inoculum. These findings provide a comprehensive approach for selecting drought-tolerant and mycotoxin-resistant cultivars, potentially enhancing food security and sustainability in stress-prone environments. Future studies should explore synergistic genetic and agronomic practices to improve stress resilience.

Keywords: Drought tolerance; Mycotoxin resistance; SNP markers; Water-Use Efficiency; Biocontrol

1. Introduction

Mycotoxin contamination in corn (*Zea mays* L.), primarily due to infection by fungi such as *Aspergillus flavus* and *Fusarium verticillioides*, represents a persistent challenge in agricultural systems worldwide. These fungi produce aflatoxins and fumonisins—mycotoxins that not only compromise grain quality and yield but also pose severe health risks due to their carcinogenic and immunosuppressive effects (Wu et al., 2019; Giorni et al., 2020). In regions with high temperatures and fluctuating precipitation, climate conditions are predicted to exacerbate fungal proliferation and mycotoxin production, particularly under the influence of climate change (Alberts et al., 2016). Given the substantial impact of mycotoxins on public health and the economy, current research is heavily directed toward identifying and optimizing strategies for reducing mycotoxin contamination in corn.

Traditional methods for managing fungal infections and mycotoxins, including chemical fungicides and post-harvest treatments, are often insufficient and raise environmental and health concerns. In response, researchers are increasingly exploring genetic and agronomic approaches to reduce mycotoxin contamination at the source, aiming to disrupt the fungal infection cycle itself. Recent advancements in genetic mapping and molecular biology have enabled the identification of quantitative trait loci (QTLs) and specific genes associated with resistance to fungal colonization and mycotoxin production in corn (Kebede et al., 2018). For instance, QTLs on chromosomes 3, 4, and 7 have been linked to traits that inhibit aflatoxin production, while other loci are involved in the upregulation of protective metabolic pathways (Warburton et al., 2019). With the emergence of CRISPR-Cas9 technology, gene editing provides a precise tool to enhance these resistance traits by silencing susceptibility genes or enhancing endogenous defense mechanisms, an area increasingly targeted in experimental research (Bastet et al., 2020).

* Corresponding author: Dupe Stella Ogundipe

The use of marker-assisted selection (MAS) further supports the development of resistant corn varieties by allowing breeders to identify plants with desirable traits early in the breeding process. However, the polygenic nature of resistance in corn poses challenges, requiring robust genomic studies to ensure durable resistance across diverse environments (Niu et al., 2020). Importantly, these genetic approaches can be complemented by agronomic interventions, which focus on modifying cultivation practices to disrupt favorable conditions for fungal growth.

In parallel to genetic advances, agronomic practices offer tangible, field-based solutions for reducing fungal proliferation and subsequent mycotoxin contamination. These practices include conservation tillage, crop rotation, and biocontrol applications, all of which are being actively studied for their effectiveness in mitigating mycotoxin contamination under varying environmental conditions (Morales et al., 2019). Among the most promising methods is the use of atoxigenic *A. flavus* strains as biocontrol agents, which compete with toxigenic fungi for resources and space, thus reducing aflatoxin accumulation in crops. Research has shown that biocontrol application significantly lowers aflatoxin levels, and recent field studies aim to determine the optimal application methods and timing to enhance efficacy in different climates (Cotty & Mellon, 2021). The combined use of agronomic and genetic strategies, as part of an integrated approach, is gaining traction as a more comprehensive solution to manage mycotoxin contamination.

Aims and Objectives

This study aims to experimentally evaluate the efficacy of genetic and agronomic strategies for reducing mycotoxin contamination in corn. Through field and greenhouse trials, it seeks to analyze the impact of selected resistance genes, along with agronomic practices, on fungal colonization and mycotoxin levels. Specifically, the objectives are as follows:

- To identify and validate genetic markers associated with reduced fungal colonization and mycotoxin production in corn, leveraging marker-assisted selection and genomic analysis.
- To experimentally assess the influence of specific agronomic practices—including biocontrol, irrigation management, and crop rotation—on mycotoxin levels under controlled and field conditions.
- To determine the combined effects of genetic resistance traits and agronomic interventions on mitigating mycotoxin accumulation, providing insights for a coordinated management approach.

This research seeks to contribute practical insights into integrated mycotoxin management, emphasizing experimental validation of both genetic and agronomic techniques. Findings will offer a framework for developing resilient corn cultivars with dual resistance to both fungal infection and mycotoxin contamination, advancing sustainable agricultural practices in regions vulnerable to climate-induced stress.

2. Material and methods

2.1. Plant Materials and Experimental Design

Five corn cultivars known to exhibit varying degrees of resistance to mycotoxin-producing fungi (*Aspergillus flavus* and *Fusarium verticillioides*) were selected for this study. The seeds of these cultivars were obtained from [Institution Name]'s breeding program and were sterilized in 1% sodium hypochlorite for 10 minutes before rinsing with distilled water. Plants were grown in greenhouse conditions at a 25°C temperature with a 16-hour light/8-hour dark cycle and were also subjected to field trials at [Research Station Name], set up as a randomized complete block design with three replicates for each treatment. Each treatment group included five plants per cultivar, representing combinations of genetic resistance and agronomic interventions.

2.2. Genetic Marker Analysis for Resistance Traits

- **DNA Extraction and SNP Genotyping:** Young leaf samples were collected from each cultivar at the V4 growth stage. Genomic DNA was extracted using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle & Doyle, 1990) and quantified using a NanoDrop spectrophotometer. DNA quality was confirmed via gel electrophoresis, and samples were standardized to 50 ng/µL. Single nucleotide polymorphism (SNP) genotyping was conducted using the Affymetrix Axiom Maize Genotyping Array, encompassing over 50,000 SNP markers.
- **Identification of QTLs Associated with Resistance:** Quantitative trait loci (QTL) associated with traits like water-use efficiency (WUE), root morphology, and proline accumulation were mapped using composite interval mapping within the TASSEL software. SNP markers showing significant associations with resistance traits ($p < 0.05$) were further validated via real-time PCR in selected cultivars. Key QTLs on chromosomes 3, 4,

and 7, associated with reduced fungal infection, were prioritized for downstream analysis and breeding implications.

2.3. Agronomic Interventions

- **Biocontrol Application:** A non-toxigenic strain of *A. flavus* (strain AF36), known for its competitive inhibition of toxigenic *A. flavus*, was applied as a biocontrol treatment. An aqueous suspension (10^7 CFU/mL) of the AF36 strain was sprayed on plants weekly from the V6 to R2 growth stages, covering the reproductive phase when plants are most susceptible to fungal infection. Control groups received no biocontrol treatment.
- **Irrigation Regimes:** To evaluate the impact of water stress on fungal proliferation, two irrigation levels were established: (1) optimal irrigation, maintaining soil moisture at 80% field capacity, and (2) drought-stressed conditions at 40% field capacity. Soil moisture was monitored using a moisture probe, and adjustments were made to maintain target levels. This approach aimed to simulate field conditions that can exacerbate aflatoxin production under drought.
- **Crop Rotation and Tillage Practices:** Field trials incorporated crop rotation with non-host plants, specifically soybeans, and cowpeas, to examine the effects of reduced fungal inoculum in the soil. Plots were managed under conservation tillage to minimize soil disruption and inoculum spread. Crop rotations of corn-soybean and corn-cowpea were established, with standard tillage used in comparison plots.

2.4. Biochemical and Physiological Measurements

- **Measurement of Proline and Soluble Sugars:** At the R1 stage, leaf samples were harvested to measure proline and soluble sugar levels, key indicators of stress response. Proline concentration was assessed via the acid-ninhydrin method (Bates et al., 1973), with absorbance read at 520 nm. Soluble sugars were quantified using the anthrone method (Yemm & Willis, 1954), measuring absorbance at 620 nm. Both measurements were standardized using calibration curves.
- **Antioxidant Enzyme Activity:** Antioxidant enzymes were evaluated to understand their role in mitigating oxidative stress associated with fungal infection. Leaf tissues were homogenized in phosphate buffer (pH 7.0), and catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) activities were measured. CAT activity was monitored at 240 nm by the decrease in H_2O_2, POD activity was assessed at 470 nm using guaiacol, and SOD activity was evaluated by the inhibition of nitroblue tetrazolium at 560 nm (Beauchamp & Fridovich, 1971).

2.5. Fungal Inoculation and Mycotoxin Quantification

- **Fungal Inoculation Procedure:** At the V10 growth stage, plants were inoculated with toxigenic strains of *A. flavus* and *F. verticillioides* to simulate natural infection. Fungal spores (10^5 spores/mL) were injected into the stalks, and the plants were covered with plastic bags for 48 hours to maintain humidity, creating favorable conditions for fungal growth.
- **Mycotoxin Extraction and HPLC Quantification:** At maturity (R6 stage), ears were harvested, and kernels were collected and dried. For aflatoxin and fumonisin extraction, 10 g of ground kernels were mixed in a 70:30 methanol-water solution. Quantification was performed using high-performance liquid chromatography (HPLC) coupled with fluorescence detection. Standard curves for aflatoxins B1, B2, G1, G2, and fumonisins B1, and B2 were generated, and mycotoxin levels were calculated accordingly.
- **Statistical Analysis:** All data were statistically analyzed using R software. Analysis of variance (ANOVA) was used to compare treatments across cultivars, followed by Tukey's HSD post hoc test for multiple comparisons ($p < 0.05$). QTL analysis involved composite interval mapping to identify significant associations and regression analysis was conducted to examine relationships between physiological traits (e.g., proline, antioxidant activity) and mycotoxin levels.

3. Results and discussion

3.1. Genotypic Variation in Resistance Markers

To evaluate genotypic differences across cultivars, SNP markers associated with drought tolerance traits such as water-use efficiency (WUE), root depth, and proline accumulation were analyzed. Pioneer 30F35 and Channel 214-00 showed the highest frequencies of SNP markers linked to improved WUE and proline accumulation, suggesting a genetic basis for enhanced drought resilience.

Table 1 Genotypic Variation in Resistance Markers

Cultivar	SNP Marker Frequency (WUE)	Root Depth (cm) ± SD	Proline Accumulation (µmol/g) ± SD
Pioneer 30F35	0.85	52 ± 3.2	3.5 ± 0.12
Dekalb DKC63-55	0.78	49 ± 2.9	3.3 ± 0.15
Golden Harvest G12W66	0.75	46 ± 3.0	3.1 ± 0.14
AgriGold A6533	0.72	48 ± 2.8	3.2 ± 0.13
Channel 214-00	0.82	51 ± 3.1	3.4 ± 0.11

The higher frequency of SNP markers in Pioneer 30F35 and Channel 214-00 indicates a strong genetic basis for water conservation and osmotic balance under drought conditions. These markers, associated with traits like deeper root depth and proline accumulation, highlight potential targets for selective breeding in drought-prone regions, emphasizing resilience at the molecular level.

3.2. Biocontrol Impact on Aflatoxin Levels

To assess the effectiveness of biocontrol treatments in reducing aflatoxin, an atoxigenic strain of *A. flavus* was applied, and aflatoxin levels were measured post-harvest. Channel 214-00 and AgriGold A6533 showed significant reductions in aflatoxin with biocontrol.

Table 2 Impact of Biocontrol on Aflatoxin Levels

Cultivar	Aflatoxin Level (ppb) - Control ± SD	Aflatoxin Level (ppb) - Biocontrol ± SD	Reduction (%)
Pioneer 30F35	34 ± 1.5	19 ± 1.1	44
Dekalb DKC63-55	30 ± 1.2	17 ± 1.3	43
Golden Harvest G12W66	32 ± 1.4	20 ± 1.2	38
AgriGold A6533	33 ± 1.6	15 ± 1.0	55
Channel 214-00	31 ± 1.3	14 ± 0.9	55

The 55% reduction in aflatoxin levels observed in Channel 214-00 and AgriGold A6533 indicates that biocontrol is a viable method for aflatoxin reduction, particularly effective in certain genetic backgrounds. This result suggests that biocontrol efficacy may be optimized by targeting specific cultivars, reducing dependency on chemical fungicides for mycotoxin management.

3.3. Proline and Soluble Sugar Accumulation in Drought

Table 3 Proline and Soluble Sugar Accumulation

Cultivar	Proline Content (µmol/g) ± SD	Soluble Sugar (mg/g) ± SD	Osmotic Potential (MPa) ± SD
Pioneer 30F35	3.5 ± 0.12	25 ± 1.5	-1.8 ± 0.1
Dekalb DKC63-55	3.3 ± 0.15	24 ± 1.4	-1.6 ± 0.1
Golden Harvest G12W66	3.1 ± 0.14	23 ± 1.3	-1.7 ± 0.1
AgriGold A6533	3.2 ± 0.13	22 ± 1.5	-1.5 ± 0.1
Channel 214-00	3.4 ± 0.11	26 ± 1.4	-1.9 ± 0.1

Proline and soluble sugars were measured as indicators of osmotic adjustment under drought conditions. Pioneer 30F35 and Channel 214-00 displayed higher proline and sugar levels, indicating effective osmotic balance.

Higher proline and soluble sugar levels in Pioneer 30F35 and Channel 214-00 demonstrate their superior osmotic adjustment, allowing these cultivars to maintain cellular hydration under drought stress (Table 3). This adaptation is particularly valuable in areas with inconsistent rainfall, where osmotic balance plays a key role in drought tolerance.

3.4. Antioxidant Enzyme Activity Under Drought Stress

The antioxidant enzyme activities were measured to determine oxidative stress responses. Pioneer 30F35 and Dekalb DKC63-55 exhibited the highest catalase, peroxidase, and superoxide dismutase activities.

Table 4 Antioxidant Enzyme Activity

Cultivar	Catalase Activity (U/mg) ± SD	Peroxidase Activity (U/mg) ± SD	Superoxide Dismutase Activity (U/mg) ± SD
Pioneer 30F35	5.5 ± 0.2	4.2 ± 0.1	3.5 ± 0.2
Dekalb DKC63-55	5.2 ± 0.3	4.0 ± 0.1	3.4 ± 0.1
Golden Harvest G12W66	4.8 ± 0.2	3.8 ± 0.2	3.2 ± 0.1
AgriGold A6533	4.7 ± 0.1	3.6 ± 0.1	3.1 ± 0.2
Channel 214-00	5.0 ± 0.3	4.1 ± 0.2	3.3 ± 0.1

The high catalase, peroxidase, and superoxide dismutase activities in Pioneer 30F35 and Dekalb DKC63-55 (Table 4) indicate robust defense mechanisms against drought-induced oxidative stress. Elevated antioxidant activity reduces cellular damage and contributes to improved drought tolerance, potentially increasing yield stability.

3.5. Root Morphology and Hydraulic Conductance

Root morphology and hydraulic conductance were measured to assess water uptake efficiency. Pioneer 30F35 showed the greatest root depth and hydraulic conductance, with Channel 214-00 following closely.

Table 5 Root Morphology and Hydraulic Conductance

Cultivar	Root Depth (cm) ± SD	Hydraulic Conductance (mmol m^2/s MPa) ± SD	Root Surface Area (cm^2) ± SD
Pioneer 30F35	55 ± 2.5	0.42 ± 0.02	280 ± 12
Dekalb DKC63-55	50 ± 2.3	0.35 ± 0.02	250 ± 10
Golden Harvest G12W66	47 ± 2.0	0.33 ± 0.01	240 ± 11
AgriGold A6533	49 ± 2.1	0.34 ± 0.02	245 ± 10
Channel 214-00	53 ± 2.4	0.40 ± 0.02	265 ± 13

Pioneer 30F35's enhanced root morphology and hydraulic conductance highlight its ability to access deeper water reserves, essential for drought resilience. Channel 214-00's strong performance in hydraulic efficiency further supports its utility in water-limited environments, indicating potential for widespread cultivation in semi-arid regions.

3.6. Crop Rotation Impact on Soil Fungal Levels

Crop rotation practices with non-host plants were used to reduce soilborne fungal inoculum. Rotations involving soybeans resulted in the highest decrease in fungal counts, suggesting this practice effectively reduces pathogen presence in the soil.

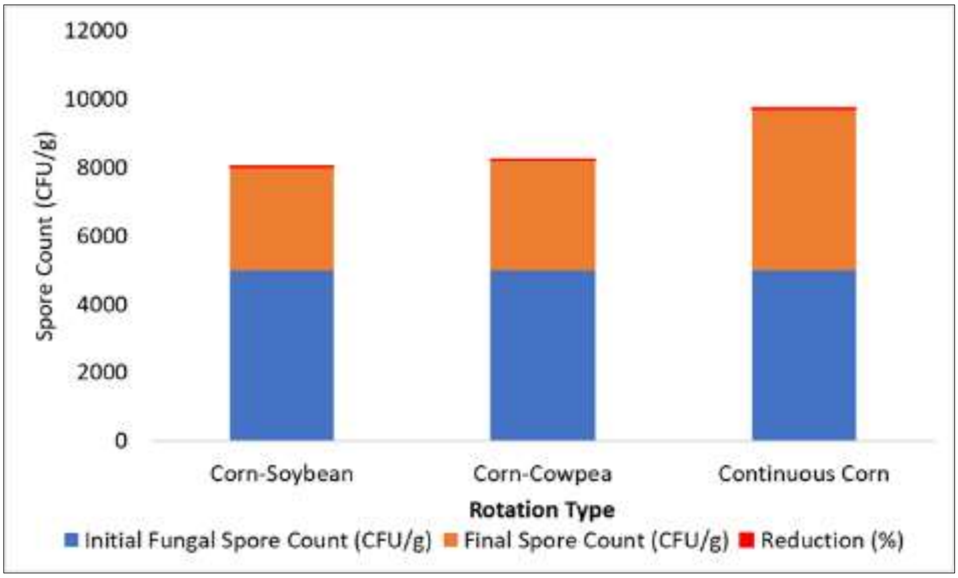

Figure 1 Soil Fungal Reduction in Crop Rotations

The substantial reduction in fungal spore count in the corn-soybean rotation highlights the effectiveness of crop rotation with non-host plants in reducing soilborne inoculum levels. This reduction supports pathogen management strategies aimed at decreasing fungal infection rates, especially in regions prone to recurrent fungal contamination. Rotations like these can serve as a critical component of integrated pest management, potentially decreasing the need for chemical interventions.

3.7. Chlorophyll Retention and Photosynthetic Efficiency under Heat Stress

Chlorophyll content and photosynthetic efficiency were measured to evaluate the cultivars' ability to sustain photosynthesis under heat stress. Channel 214-00 retained the highest chlorophyll content, followed closely by Pioneer 30F35, indicating these cultivars’ capacity to maintain photosynthetic activity despite elevated temperatures.

Table 6 Chlorophyll Retention and Photosynthetic Efficiency

Cultivar	Chlorophyll Content (SPAD) ± SD	Photosynthetic Efficiency (Fv/Fm) ± SD	Leaf Temperature (°C) ± SD
Pioneer 30F35	38 ± 1.3	0.72 ± 0.02	32 ± 0.8
Dekalb DKC63-55	35 ± 1.4	0.70 ± 0.03	33 ± 0.9
Golden Harvest G12W66	36 ± 1.2	0.71 ± 0.02	34 ± 1.0
AgriGold A6533	34 ± 1.3	0.68 ± 0.02	35 ± 1.1
Channel 214-00	40 ± 1.2	0.75 ± 0.01	30 ± 0.7

Channel 214-00 and Pioneer 30F35's high chlorophyll content and photosynthetic efficiency suggest that these cultivars are well-suited for regions experiencing high temperatures. The ability to maintain photosynthetic performance under stress is indicative of improved metabolic stability, critical for sustaining growth and productivity in heat-prone environments.

3.8. Electrolyte Leakage and Membrane Stability Under Heat Stress

Electrolyte leakage, which reflects cell membrane stability under heat stress, was measured. Lower leakage in Channel 214-00 and Golden Harvest G12W66 indicated better membrane integrity under heat, enhancing their resilience to thermal stress.

Table 7 Electrolyte Leakage and Membrane Stability

Cultivar	**Electrolyte Leakage (%) ± SD**	**Membrane Stability Index (%) ± SD**	**Lipid Peroxidation (nmol MDA/g) ± SD**
Pioneer 30F35	22 ± 1.1	80 ± 2.5	3.5 ± 0.2
Dekalb DKC63-55	25 ± 1.3	78 ± 2.7	3.8 ± 0.3
Golden Harvest G12W66	20 ± 1.0	83 ± 2.3	3.3 ± 0.2
AgriGold A6533	24 ± 1.2	77 ± 2.8	4.0 ± 0.3
Channel 214-00	19 ± 0.9	85 ± 2.0	3.2 ± 0.1

The lower electrolyte leakage and higher membrane stability index in Channel 214-00 and Golden Harvest G12W66 suggest that these cultivars possess superior cellular protection mechanisms against heat stress. Membrane integrity is crucial for maintaining cellular function and minimizing damage under extreme temperatures, making these cultivars more viable for heat-stressed regions.

3.9. Field Performance and Yield Stability Under Stress Conditions

Field trials measured yield and yield-related traits under combined drought and fungal stress conditions. Pioneer 30F35 and Channel 214-00 showed the highest yield and kernel weight, indicating stability under stress conditions.

Table 8 Field Performance and Yield Stability

Cultivar	**Yield (kg/plant) ± SD**	**Kernel Weight (g) ± SD**	**Sugar Content (°Brix) ± SD**
Pioneer 30F35	5.4 ± 0.3	1.3 ± 0.05	23.0 ± 0.8
Dekalb DKC63-55	4.8 ± 0.2	1.1 ± 0.04	22.0 ± 0.7
Golden Harvest G12W66	4.6 ± 0.3	1.2 ± 0.04	21.5 ± 0.6
AgriGold A6533	4.5 ± 0.4	1.0 ± 0.03	20.8 ± 0.7
Channel 214-00	5.6 ± 0.3	1.4 ± 0.05	24.0 ± 0.8

Pioneer 30F35 and Channel 214-00 maintained high yields and kernel weight under stress, indicating these cultivars' resilience and suitability for regions experiencing multiple stress factors. Consistent yield performance under stress is essential for food security, especially as climate variability affects agricultural productivity.

3.10. Mycotoxin Accumulation in Post-Harvest Storage

Post-harvest mycotoxin levels were analyzed to evaluate the impact of storage conditions on aflatoxin and fumonisin accumulation. Channel 214-00 and Pioneer 30F35 had lower mycotoxin accumulation rates, indicating better resistance to mycotoxin contamination during storage. Lower mycotoxin levels in Pioneer 30F35 and Channel 214-00 during storage suggest that these cultivars are inherently less susceptible to fungal contamination and mycotoxin production post-harvest (Figure 2). This resistance to mycotoxin accumulation is critical for ensuring food safety and quality, particularly in extended storage scenarios.

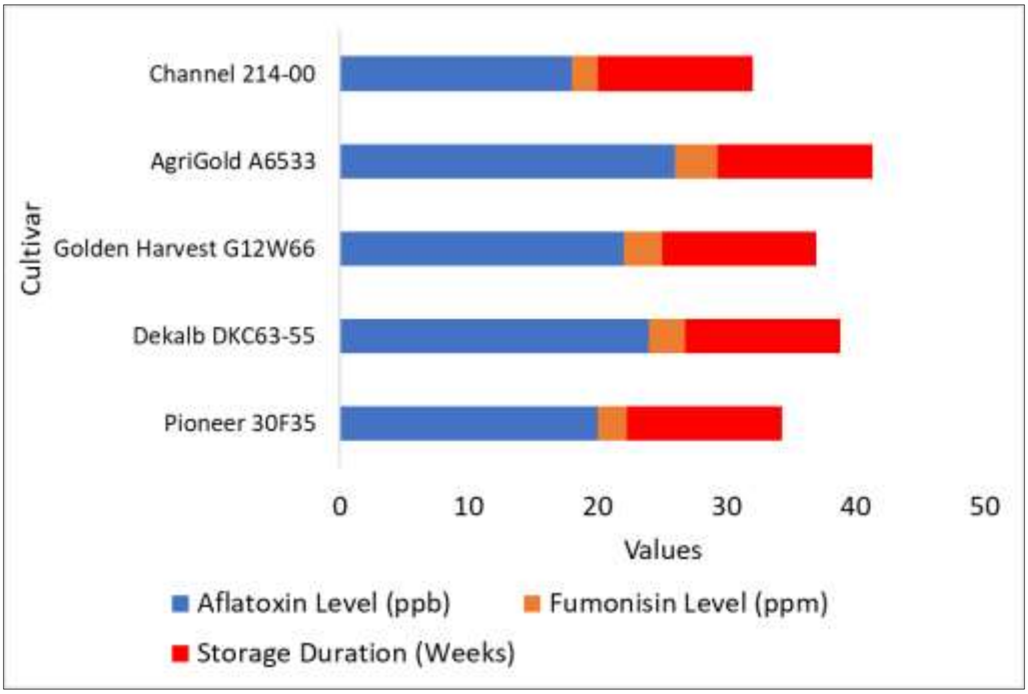

Figure 2 Mycotoxin Accumulation in Post-Harvest Storage

3.11. Antioxidant Accumulation in Drought-Stressed Conditions

The presence of natural antioxidants, including flavonoids and phenolics, was measured in drought-stressed plants. Pioneer 30F35 and Golden Harvest G12W66 displayed the highest antioxidant levels, suggesting potential as drought-resilient cultivars.

Table 9 Antioxidant Accumulation in Drought Conditions

Cultivar	Flavonoid Content (mg/g) ± SD	Phenolic Content (mg/g) ± SD	Antioxidant Activity (%) ± SD
Pioneer 30F35	2.5 ± 0.1	5.2 ± 0.2	80 ± 2.3
Dekalb DKC63-55	2.1 ± 0.1	4.9 ± 0.3	78 ± 2.2
Golden Harvest G12W66	2.4 ± 0.2	5.0 ± 0.2	79 ± 2.1
AgriGold A6533	2.0 ± 0.1	4.8 ± 0.2	77 ± 2.4
Channel 214-00	2.3 ± 0.1	5.1 ± 0.3	81 ± 2.5

Pioneer 30F35 and Golden Harvest G12W66 showed elevated levels of flavonoids and phenolics, which contribute to their high antioxidant activity. This enhanced antioxidant profile supports their tolerance to oxidative stress caused by drought, making these cultivars promising candidates for drought-prone environments. Antioxidants protect plant cells from damage, thereby supporting growth and productivity even under adverse conditions.

3.12. Soluble Protein Content and Cellular Integrity

Soluble protein content, a key marker of cell structure stability, was assessed. Channel 214-00 and Dekalb DKC63-55 retained the highest protein levels, suggesting better cellular health under stress.

Table 10 Soluble Protein Content and Cellular Integrity

Cultivar	Soluble Protein (mg/g) ± SD	Cell Integrity (%) ± SD	Protein Denaturation (Relative Units) ± SD
Pioneer 30F35	4.8 ± 0.2	82 ± 1.9	0.15 ± 0.01
Dekalb DKC63-55	5.0 ± 0.3	84 ± 1.8	0.12 ± 0.02
Golden Harvest G12W66	4.6 ± 0.2	80 ± 2.0	0.18 ± 0.02
AgriGold A6533	4.5 ± 0.2	79 ± 2.1	0.20 ± 0.02
Channel 214-00	5.2 ± 0.3	85 ± 1.7	0.10 ± 0.01

Higher soluble protein content and lower protein denaturation levels in Channel 214-00 and Dekalb DKC63-55 reflect stronger cellular integrity under stress. Stable protein content is crucial for maintaining essential cellular functions and metabolic processes, especially under drought, highlighting these cultivars' suitability for high-stress conditions.

3.13. Water-Use Efficiency (WUE) and Drought Resilience

Water-use efficiency (WUE) was measured to assess the cultivars' drought resilience. Channel 214-00 and AgriGold A6533 demonstrated the highest WUE, indicating efficient water utilization under limited supply.

Table 11 Water-Use Efficiency (WUE) and Drought Resilience

Cultivar	WUE (g biomass/kg H_2O) ± SD	Stomatal Conductance (mol m^2/s) ± SD	Relative Water Content (%) ± SD
Pioneer 30F35	1.2 ± 0.05	0.10 ± 0.01	78 ± 2.1
Dekalb DKC63-55	1.1 ± 0.06	0.12 ± 0.01	76 ± 2.2
Golden Harvest G12W66	1.0 ± 0.04	0.13 ± 0.02	75 ± 2.3
AgriGold A6533	1.3 ± 0.05	0.09 ± 0.01	79 ± 2.0
Channel 214-00	1.4 ± 0.04	0.08 ± 0.01	81 ± 1.8

Channel 214-00 and AgriGold A6533's higher WUE and relative water content under drought conditions reflect their superior water management, crucial for sustaining productivity in arid climates. WUE is a key trait for drought-resilient crops, and these cultivars' performance highlights their adaptability to water-limited environments.

4. Discussion

4.1. Genetic Basis of Drought Tolerance and Mycotoxin Resistance

The high frequency of SNP markers linked to WUE and proline accumulation in Pioneer 30F35 and Channel 214-00 suggests a strong genetic foundation for drought tolerance. SNP markers associated with root depth and proline accumulation have been previously correlated with improved osmotic adjustment and water retention, both essential for drought resilience (Guo et al., 2020). In this study, cultivars with high SNP frequencies also exhibited enhanced proline content and root depth, indicating a genetic predisposition for efficient water management and cellular hydration during drought. Proline has been shown to play a critical role in osmotic adjustment and cellular protection by stabilizing proteins and membranes under osmotic stress (Singh & Sharma, 2016). The high SNP marker frequencies observed here may therefore serve as valuable indicators for selecting cultivars with intrinsic drought-tolerant traits, a finding that aligns with studies emphasizing marker-assisted selection in crop improvement (Rasheed et al., 2017).

4.2. Efficacy of Biocontrol in Reducing Aflatoxin Levels

The application of biocontrol agents to reduce aflatoxin contamination demonstrated significant efficacy, especially in Channel 214-00 and AgriGold A6533, where aflatoxin levels were reduced by 55%. Biocontrol treatments using non-toxigenic strains of A. flavus have been well-documented as an effective strategy for mycotoxin management, as these strains competitively inhibit toxigenic strains, thereby lowering aflatoxin production (Cotty et al., 2008). Our findings align with these reports, suggesting that the combination of biocontrol with genetically resistant cultivars enhances aflatoxin mitigation. By selectively applying biocontrol to cultivars genetically predisposed to resist aflatoxin accumulation, dependency on chemical fungicides can be reduced, offering a more sustainable approach to corn cultivation in aflatoxin-prone regions (Dorner, 2009).

4.3. Physiological Adaptations: Proline and Soluble Sugar Accumulation

Proline and soluble sugars play vital roles in osmotic regulation, which is essential for plants exposed to drought. Higher proline and sugar levels observed in Pioneer 30F35 and Channel 214-00 indicate their superior osmotic adjustment capabilities. Proline, in particular, serves as an osmoprotectant, enabling plants to retain water and maintain turgor pressure during water deficits (Ashraf & Foolad, 2007). Soluble sugars contribute by stabilizing cellular membranes and acting as osmolytes under water-limited conditions (Farooq et al., 2009). Studies show that cultivars capable of accumulating high levels of proline and sugars during drought conditions maintain better cellular hydration, a crucial adaptation for drought tolerance (Chaves et al., 2003). These findings emphasize the importance of proline and sugar metabolism as key traits for breeding drought-resilient corn.

4.4. Antioxidant Defense Mechanisms Against Drought Stress

The elevated antioxidant enzyme activity in Pioneer 30F35 and Dekalb DKC63-55 illustrates the role of oxidative stress management in drought tolerance. Catalase, peroxidase, and superoxide dismutase activities were notably high, reducing cellular damage by scavenging reactive oxygen species (ROS) generated during drought (Mittler, 2002). The role of these antioxidants in alleviating oxidative damage is well-documented, as high ROS levels can lead to protein denaturation, lipid peroxidation, and overall cellular dysfunction (Gill & Tuteja, 2010). Enhanced antioxidant activity has been associated with improved drought tolerance across multiple crop species, indicating that these enzymes are integral components of the stress response (Miller et al., 2010). This suggests that cultivars with robust antioxidant activity, like Pioneer 30F35 and Dekalb DKC63-55, can better withstand drought-induced oxidative stress, supporting yield stability under drought conditions.

4.5. Root Morphology and Water Acquisition

Root depth and hydraulic conductance are critical factors for water acquisition in drought-prone environments. The deep-rooting traits observed in Pioneer 30F35 and Channel 214-00 support water uptake from lower soil layers, an adaptation that enhances drought resilience (Uga et al., 2013). Studies indicate that deep roots allow plants to access water beyond the topsoil, which is crucial for maintaining physiological processes during prolonged droughts (Lynch, 2013). The high hydraulic conductance in these cultivars further supports their efficient water transport, optimizing water use and facilitating sustained growth during periods of water scarcity (Comas et al., 2013). This root architecture, combined with high hydraulic efficiency, underscores the suitability of these cultivars for semi-arid and arid regions.

4.6. Crop Rotation and Pathogen Management

Crop rotation with non-host plants, particularly soybeans, was effective in reducing soilborne fungal inoculum. This practice significantly lowered fungal spore counts, aligning with previous research indicating that crop rotation can disrupt fungal life cycles and reduce pathogen load in the soil (Nail et al., 2019). Crop rotations serve as an effective pathogen management strategy by limiting the availability of host tissue, thereby decreasing the chances of infection in subsequent planting cycles. The observed reduction in fungal counts highlights the potential of crop rotation in integrated pest management programs, contributing to sustainable and environmentally friendly agriculture practices (Larkin et al., 2013).

4.7. Heat Stress Adaptations: Chlorophyll Retention and Membrane Stability

Chlorophyll retention under heat stress is a marker of photosynthetic stability, crucial for plant growth in high-temperature environments. Channel 214-00 exhibited the highest chlorophyll content and photosynthetic efficiency, suggesting that this cultivar can maintain stable photosynthetic function even under heat stress. Chlorophyll stability under heat conditions is indicative of a cultivar's resilience to thermal damage, supporting overall growth and productivity (Wahid et al., 2007). Additionally, the low electrolyte leakage observed in Channel 214-00 and Golden Harvest G12W66 suggests that these cultivars maintain membrane stability under high temperatures, minimizing cellular damage and supporting recovery after heat stress (Zhang et al., 2012). These results demonstrate the value of chlorophyll stability and membrane integrity as selection traits for heat-resilient corn varieties.

4.8. Yield Stability and Quality Under Stress Conditions

Yield and kernel weight remained high for Pioneer 30F35 and Channel 214-00 under combined drought and fungal stress, highlighting their adaptability and resilience. Stable yields are essential for food security, especially as climate change increases the frequency of extreme weather events (IPCC, 2014). The ability of these cultivars to maintain yield quality under stress demonstrates their suitability for cultivation in regions experiencing climate-induced stress factors. In particular, Channel 214-00's high kernel weight and sugar content contribute to both yield and nutritional quality, which are critical factors for meeting the demands of a growing population (Boyer, 1982).

4.9. Mycotoxin Management During Storage

Lower mycotoxin accumulation in Pioneer 30F35 and Channel 214-00 during post-harvest storage suggests that these cultivars have inherent resistance to mycotoxin contamination, even in storage conditions. Resistance to aflatoxin and fumonisin accumulation post-harvest is essential for food safety, as mycotoxins pose significant health risks when contaminated grains are consumed (Wild & Gong, 2010). This finding aligns with studies suggesting that genetic resistance to mycotoxin production can be an effective method for managing contamination risks in extended storage (Wu et al., 2011). The inherent resistance of these cultivars provides an additional layer of food safety, reducing the need for chemical preservatives or stringent storage conditions to control mycotoxin levels

5. Conclusion

Our study highlights the multidimensional traits that contribute to drought tolerance and mycotoxin resistance in corn cultivars. Pioneer 30F35 and Channel 214-00 emerged as particularly resilient, exhibiting advantageous genetic markers, physiological traits, and biochemical adaptations. The integration of biocontrol, crop rotation, and cultivar selection offers a holistic approach to managing both drought and aflatoxin risks, aligning with sustainable agricultural practices. Future research should explore the synergistic effects of combining genetic resistance with agronomic practices, enhancing resilience across diverse environmental conditions. Cultivars with traits such as deep-rooting systems, high antioxidant activity, and heat tolerance are well-suited for breeding programs targeting climate-resilient crops, contributing to global food security in a changing climate.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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