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**STUDY OF GENETIC AND AGROTECHNICAL FACTORS ON MAIZE YIELD  
AND MYCOTOXINS CONTAMINATION**

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2025

**STUDY OF GENETIC AND AGROTECHNICAL FACTORS ON MAIZE YIELD  
AND MYCOTOXIN CONTAMINATION**

A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of Doctor  
of Philosophy in Crop Production and Horticultural Science

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The date and venue of the dissertation defense: .....,2025, Debrecen, Hungary

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## LIST OF ABBREVIATIONS

AFB1	Aflatoxin B1
AF%	Percentage of Kernels Damaged by <i>A. flavus</i>
CAST	Council for Agricultural Science Technology
DON	Deoxynivalenol
EC	European Commission
FAO	Food and Agriculture Organisation of the United Nations
FAOstat	Food and Agriculture Organization Statistics
FBS	Fumonisin B1+B2+FB3 (Total fumonisins)
FG%	Percentage of Kernels Damaged by <i>F. graminearum</i>
FV%	Percentage of Kernels Damaged by <i>F. verticillioides</i>
Genstat	General Statistics
GK	Gabonakutató (Cereal Research Institute)
IGC	International Grain Council
LAI	Leaf Area Index
LSD	Least significance Differences
LOD	Limit of detection
MTL	Maximum Tolerable levels
N	Nitrogen
NDVI	Normalized Difference Vegetation Index
NIR	Near Infrared
SPAD	Soil Plant Analysis Development Index
SSA	Sub-Saharan Africa
V6	Vegetative stage 6 - Sixth leaf collar visible
VPD	Vapor Pressure Deficit
VT	Vegetative Tasseling Growth Stage- The last branch of tassel visible
QTLs	Quantitative Traits Loci
R0-R3	Anthesis-Visible silks-Blister stage/Clear liquid in kernel-Milk stage/White milky fluid in kernels
R6	Maturity stage – Black layer visible at the base of grain
RILs	Recombinant Inbred Lines

# **1. INTRODUCTION**

## **1.1 Background and rationale**

In global agricultural food systems, maize (*Zea mays* L.) has become crucial for food, feed, and industrial raw materials. Over recent decades, maize production has dramatically increased due to rising demand, technological advancements, area expansion, and improved yields (Erenstein et al., 2022). In the past decade, maize production surpassed one billion metric tons, ahead of rice (751 million metric tons) and wheat (747 million metric tons). These three cereals account for about 90% of global primary cereal production, providing approximately 42% of calories and 37% of protein in global diets (FAOStat, 2023). It was noted that maize was the most produced cereal in 2019, 2020, and 2022, with its grain oilseed index (GOI) sub-index rising by 37%, significantly above wheat's 7%. ( IGC, 2022) Maize is projected to be the most traded cereal due to its versatility (Erenstein et al., 2022). In SSA, South America, and parts of Asia, maize is vital for human consumption, providing about 20% of food calories (Shiferaw et al., 2011). As an industrial and energy crop, maize's diverse role is essential for global food systems and nutrition security.

Climate variability and land degradation significantly impact global crop production, threatening food security. Research has consistently highlighted the prevalence of climate change, indicating that alterations in precipitation patterns and increased weather variability are key consequences (Böhm et al., 2010; Hansen et al., 2010; Rohde et al., 2013; Parry, 2007; Mann et al., 2009; Screen and Simmonds, 2010). Extreme weather events like heat waves, droughts, and heavy rainfall are becoming more common (Pachauri and Reisinger, 2007). Reports show that the global mean temperature has risen by 0.8°C since the mid-19th century, backed by independent datasets covering land, seas, and ocean surface temperatures (Solomon, 2007). Climate change causes are anthropogenic and natural (Wheeler and Braun, 2013). The predictions indicate that average rainfall is expected to rise in polar areas and certain wet mid-latitudes, while declines are projected in arid mid-latitude and subtropical regions, increasing drought risk (Medina et al., 2017). Consequently, extreme weather events significantly threaten global crop production and food security (Lobell et al., 2013).

The productivity of maize as a crop will be significantly influenced by the effects of climate change (Wheeler and Braun, 2013). Plant pathogens, disease-spreading pests, and host-pathogen interactions will further impact crop quality due to shifting climate conditions

(Donatelli et al., 2017). Land degradation reduces soil fertility, hindering production. Fertilization optimizes nutrient availability, addressing deficiencies for healthy crop growth. Nitrogen is crucial for grain crop productivity and is a major limiting factor. It impacts maize growth by enhancing leaf area and photosynthesis (Geith et al., 2022), directly influencing yield and grain quality (Gao et al., 2020). On food security, the alterations in plant pathogens, disease-spreading pests, and host-pest interactions compromise food quality. Mycotoxin contamination produced by fungi poses significant food safety risks that are expected to be affected by climate change (Miraglia et al., 2009); hence, serious attention is needed.

Maize contamination by mycotoxin is alarming despite the economic importance of maize worldwide. About 25% of maize was reported to exhibit contamination with mycotoxins at varying concentrations, positioning mycotoxin contamination as a significant global issue related to food safety and public health (James and Zikankuba, 2018). Public-private partnerships and non-governmental organizations (NGOs) are actively engaged in ongoing research and outreach initiatives targeting the multifaceted issues associated with the prevention and mitigation of mycotoxins. These initiatives encompass various comprehensive global projects, notably funded by the European Commission, such as MycoGlobe (2004-2008), MycRed (2012-2016), MyToolBox (2016-2020), and MycoKey (2016-2020) ([www.mycokey.eu](http://www.mycokey.eu)). The primary objective of these projects is to develop and assess integrated, sustainable solutions and innovative methodologies aimed at substantially reducing mycotoxin levels in food and feed chains of economic significance. Additionally, they emphasize the creation and application of rapid, reliable, and validated strategies for the detection of toxigenic fungi (Logrieco et al., 2021). *Fusarium graminearum*, which produces deoxynivalenol (DON) and zearalenone (ZEA); *Fusarium verticillioides*, producing fumonisins toxins; and *Aspergillus flavus*, which produces aflatoxins comprises three main fungi infecting maize. Mycotoxins contaminate maize kernels and are mainly brought on by preharvest fungal infections in the field and throughout the production chain as conditions are favorable (Xu et al., 2021; Matumba et al., 2021).

The prevention of mycotoxins hinges on managing mold-producing fungi, which necessitates implementing strategies both before and after the harvest (Mielniczuk and Skwaryło-Bednarz, 2020). It is crucial to identify the factors that favor fungal proliferation and subsequent mycotoxin synthesis, including environmental variables like weather

patterns, soil nutrient levels, drought, pest infestations, or unexpected rainfall during the harvesting period (Degraeve et al., 2016) and the tolerance levels of the hybrids (Mesterházy et al., 2020). Additionally, the contamination of maize with mycotoxins is significantly affected by agronomic practices employed in the cultivation process (Ariño et al., 2009; Bocianowski et al., 2020).

Finding resistant maize genotypes has been the primary tactic for combating the issue (Hawkins et al., 2015). Study for fungi-resistant germplasm has been the focus of numerous studies across the globe (Logrieco et al., 2021; Brown et al., 2016; Williams et al., 2015; Mayfield et al., 2012; Williams et al., 2008; Williams and Windham, 2006; Williams and Windham, 2001; Scott and Zummo, 1992, 1990). However, commercial maize hybrids tolerance information is rarely disclosed to users, although they are subjected to varying levels of sensitivity to infection by toxins-producing fungi (Mesterházy et al., 2022). Screening of tolerance to some commercial hybrids has been conducted by some studies (Mesterházy et al., 2022; Zafar et al., 2021; Szabo et al., 2018), indicating tolerance levels influence a significant reduction and/or increase in the severity of diseases and subsequent mycotoxin production suggesting for choice of less susceptible genotypes.

On the other hand, the role of agrotechnical factors and their impacts on stress conditions has been explored. Biotic and abiotic stresses aggravate plant susceptibility and accelerate disease development (Bostok et al., 2014). Blandino et al., 2008 explain the increased maize susceptibility to infection by microorganisms is more severe if exposed to stresses such as drought and fertility. Maize crops under stressful circumstances are frequently related to the high occurrence of maize diseases brought on by toxin-producing fungi on stalks and ears (Miller, 2001; Munkvold, 2003).

Plant nutrition, especially adequate nitrogen (N) fertilization, is crucial for plants' growth and development and affects grain yield and quality. Imbalances of nitrogen affect grain quality and are aggravated by its influence on fungal colonization and mycotoxin contamination in maize. Studies show conflicting data on its impact on various mycotoxins (Scarpino et al., 2022). Therefore, balancing nitrogen levels in soil and avoiding deficiencies or excesses is crucial for managing mycotoxin risks (Wilson et al., 2005), as nutrient imbalances can aggravate susceptibility to pests and diseases (Tubajika et al., 1999).

The coexistence of host susceptibility (tolerance level of the hybrids) and environmental factors that favor fungal infection, development, and toxin production determines mycotoxin contamination in maize. Other factors, such as the agrotechnical influence, aggravate the contamination levels. Nitrogen fertilization is crucial in influencing crop yield and quality and affecting fungal colonization and the microclimate associated with the crop (Doohan et al., 2003; Nicholson et al., 2004). Therefore, finding high-yielding hybrids with higher tolerance levels and improved stability to fungal colonization and mycotoxin contamination in varying weather conditions across years sets the basis of this study.

## **1.2 Objectives**

The present study was designed to comprehend the interactive influence of N fertilizer application and the susceptibility of maize hybrids on fungal colonization and the mycotoxin content of grains with the primary aim of assessing how hybrid genotypes can influence nitrogen fertilization in maize concerning fungal colonization and disease development as well as the resulting mycotoxins contaminations. The data for yield, grain quality, progression of fungi ear rot disease, and mycotoxin contamination across the years enabled this study to address the objectives;

- 1) Effects of yearly variation in agrometeorological conditions on yield and mycotoxin contamination of selected maize hybrids under nitrogen treatment
- 2) Assessing the susceptibility of selected maize hybrids to fungal colonization and mycotoxin contamination under nitrogen treatments in varying weather conditions across crop cultivation years.
- 3) Evaluating nitrogen fertilization effects on yield, grain quality, and mycotoxin contamination of selected maize hybrids
- 4) Evaluating implications of relative chlorophyll and canopy reflectance indices for yield and grain quality, fungal diseases development, and mycotoxin contamination of maize hybrids under nitrogen treatments

## 2. LITERATURE REVIEW

### 2.1 Global maize production overview

Maize has emerged as the most extensively produced cereal globally, surpassing wheat and rice in the past two decades (Table 1). Over the last decade, maize production has experienced significant growth, exceeding one billion metric tons, representing nearly a twofold increase compared to levels observed over the previous three decades. This surge in productivity can be attributed to the increased adoption of improved hybrid varieties, increased utilization of intensive agricultural inputs (Eresntein et al., 2022), and the expansion of maize-cultivated areas ( Table 1).

*Table 1:* Global maize production trends (FAOStat, 2024)

Duration years	Area harvested (Million Ha)			Yield (Tons/ha)			Production ( Million tons)		
	Maize	Wheat	Rice	Maize	Wheat	Rice	Maize	Wheat	Rice
1961-1970	109	214	125	2.18	1.30	2.11	238	278	265
1971-1980	122	225	140	2.89	1.72	2.52	352	388	352
1981-1990	128	229	145	3.48	2.22	3.22	447	509	466
1991-2000	137	220	150	4.07	2.59	3.73	558	570	560
2001-2010	151	217	154	4.82	2.87	4.12	731	622	636
2011-2020	190	218	163	5.53	3.36	4.58	1054	733	744

The dynamics of maize production over the past 30 years reflect earlier historical trends. The global area dedicated to maize cultivation has seen a nearly two-fold increase since the 1960s, rising from 109 million hectares (1961-1970) to approximately 190 million hectares (2011-2020), representing an increase of 75%. This area expansion has notably accelerated since the early 2000s (Figure 1 A). Given the relative stagnation in wheat cultivation area, current projections suggest that maize will likely surpass wheat as the predominant crop by 2030 (Erenstein et al., 2021). Since 1961, global maize yields have experienced a significant increase, nearly three-fold from 2 tons per hectare (1961-1970) to the present rate of 5.5 tons per hectare (2011-2020), which represents a growth of 175% (Fig. 1 C). Consequently, maize production has raised five-fold over the same period, reflecting an overall increase of 342% ( Fig. 1 B).

Maize, classified as a C4 plant, demonstrates high photosynthetic efficiency and adaptability across diverse environments in warmer and dryer conditions than C3 plants, including tropical, subtropical, and temperate regions (Erenstein et al., 2021). Mekonnen and Gerbens-Leenes (2020) report that a kilogram of maize produce requires approximately 1222 liters of water, which is advantageous against other staple cereals. In terms of nutritional energy,

maize exhibits the most efficient water footprint per kilocalorie of energy consumed, requiring only 0.41 liters of water /kcal. Despite its efficiency, maize accounts for 6% of the worldwide unsustainable blue water footprint, (Mekonnen and Gerbens-Leenes, 2020).

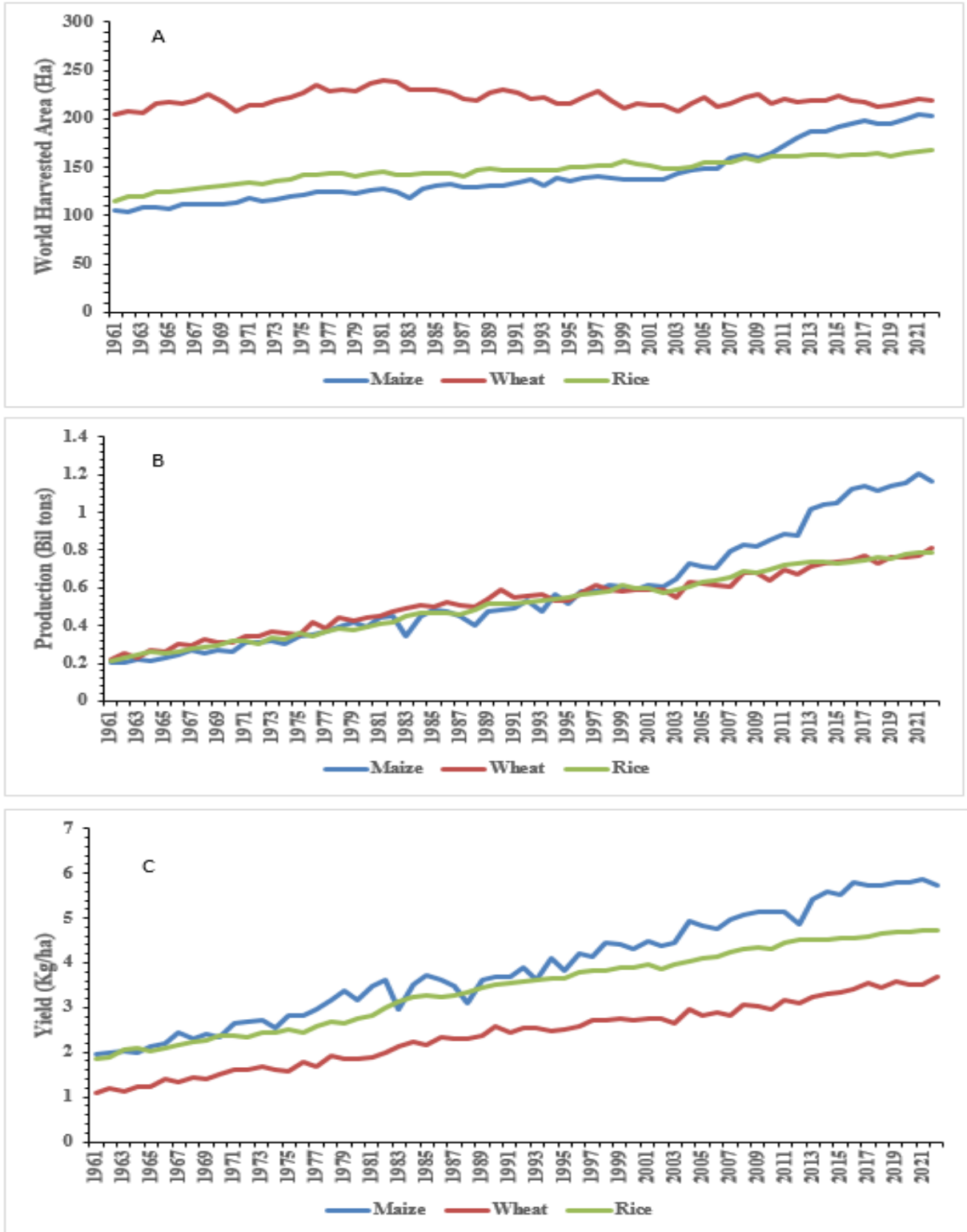


Figure 1: Changes in global maize production trends compared to other staple cereals (FAOSTAT, 2024)

The diverse agroecological environments for maize cultivation, ranging from wet to dry and varying altitudes, are affected by climatic conditions. These variations affect the production of maize, including hybrid performance that is specifically developed to fit the growing season conditions based on temperature and rainfall (Aakash et al., 2022). Considering annual weather variability, particularly rainfall (water availability), is imperative because over 50% of the Earth's land depends on rainfall for agriculture, with around 80% of maize being grown under rainfed conditions (Aakash et al., 2022). In this context, selecting crop variety and managing nitrogen levels influence overall production outcomes.

## **2.2 Maize production and mycotoxin contamination**

### **2.2.1 Prevalence of mycotoxins in cereals and their causal agents**

Studies reveal incidences of mycotoxins in foods and feed from many parts of the world (Table 2). They are currently considered one of the most hazardous and risky contaminants of the world's agricultural commodities (Kumar et al., 2020). They contaminate the commodities throughout their production chains. Cereals are the most significant food source in many countries (Pimentel, 2009), and crops are among the most commonly contaminated commodities (Tola and Kebede, 2016).

Mycotoxins dominant in cereals can be grouped according to the fungi genera producing each respective toxins (Munkvold et al., 2019; Tola and Kebede, 2016). *Fusarium*, *Aspergillus* and *Penicillium* are major toxigenic fungi in cereals (Pinton and Oswald, 2014; Munkvold et al., 2019; Kumar et al., 2021). Subsequently, since fungi produce them, mycotoxins are associated with diseases and/or moldy to infested crops. Several species of the named fungal genera are globally essential pathogens of cereals causing disease development.

*Fusarium* causes two distinctive diseases in maize ear in the field, which are Gibberella ear rot (GER) caused by *Fusarium graminearum* and Fusarium ear rot (FER) caused by *F. moniliforme* or *F. verticillioides* (Mesterházy et al., 2012; Munkvold et al., 2019). The fungi are responsible for the development of Fusarium head blight (FHB), seedling light, crown rot, and root rot in wheat (Nicholson et al., 2004; Peraldi et al., 2011; Mesterházy et al., 2018).

**Table 2: Mycotoxigenic Fungi species associated with cereal crops and their mycotoxins**

<b>Fungi species (Cause agent)</b>	<b>Mycotoxin(s) produced</b>
<b><i>Aspergillus Species</i></b>	
<i>A. alliaceus</i>	Ochratoxins, Penicillic Acid
<i>A. chevalieri</i>	Sterigmatocystin,
<b><i>A. flavus</i></b>	<b>Aflatoxins</b> , Cyclopiazonic Acid, Sterigmatocystin
<i>A. glaucus</i>	Ochratoxins
<i>A. nomius</i>	Aflatoxins
<i>A. ochraceus</i>	Ochratoxins, Penicillic Acid
<i>A. parasiticus</i>	Aflatoxins, Sterigmatocystin
<i>A. rubrobrunneus</i>	Sterigmatocystin
<i>A. sulphureus</i>	Ochratoxins, Penicillic Acid
<i>A. sydowii</i>	Sterigmatocystin
<i>A. pseudotamarii</i>	Aflatoxins, Cyclopiazonic Acid
<i>A. ustus</i>	Sterigmatocystin
<i>A. versicolor</i>	Cyclopiazonic Acid ,Sterigmatocystin ,Versicolorins
<b><i>Penicillium Species</i></b>	
<i>P. chrysogenum</i>	Ochratoxins,
<i>P. citrinum</i>	Citrinin
<i>P. cyclopiumb</i>	Ochratoxins, Penicillic Acid
<i>P. expansum</i>	Citrinin, Penicillic Acid
<i>P. purpurogenumd</i>	Rubratoxins
<i>P. urticae</i>	Penicillic Acid
<i>P. variabile</i>	Ochratoxins,
<i>P. verrucosumf</i>	Ochratoxins, Citrinin, Penicillic Acid
<i>P. viridicatum</i>	Ochratoxins, Citrinin, Penicillic Acid
<b><i>Fusarium Species</i></b>	
<i>Fusarium acuminatum</i>	Aurofusarin, Beauvericin, Chlamydosporo, Enniatins, Fusarins, Moniliformin, Trichothecenes, Diacetoxyscirpenol, HT-2, T-2
<i>Fusarium armeniacum</i>	Beauvericin, Fusarins, Trichothecenes, HT-2, T-2
<i>Fusarium avenaceum</i>	Aurofusarin, Beauvericin, Chlamydosporo, Enniatins Fusarins, Moniliformin,
<i>Fusarium boothii</i>	Trichothecenes, Deoxynivalenol
<i>Fusarium chlamydosporum</i>	Chlamydosporol, Enniatins, Moniliformin,
<i>Fusarium cortaderiae</i>	Trichothecenes, Nivalenol
<i>Fusarium crookwellense</i>	Aurofusarin, Butenolide, Culmorin, Cyclonerodiol, Fusaric acid, Fusarins, Trichothecenes, Nivalenol, Zearalenone
<i>Fusarium culmorum</i>	Aurofusarin, Butenolide, Chlamydosporol, Culmorin, Cyclonerodiol, Enniatins, Fusarins, Moniliformin, Trichothecenes, Deoxynivalenol, Nivalenol, Zearalenone
<i>Fusarium dlamini</i>	Beauvericin, Fumonisin, Moniliformin
<i>Fusarium equiseti</i>	Beauvericin, Moniliformin, Trichothecenes, Diacetoxyscirpenol, Nivalenol, T-2, Zearalenone
<i>Fusarium globosum</i>	Beauvericin, Fumonisin, Fusaproliferin
<b><i>Fusarium graminearum</i></b>	Aurofusarin, Butenolide, Chlamydosporol, Culmorin, Cyclonerodiol, Fusarins, Trichothecenes, <b>Deoxynivalenol</b> , Nivalenol, Zearalenone
<i>Fusarium meridionale</i>	Trichothecenes, Nivalenol
<i>Fusarium oxysporum</i>	Beauvericin, Enniatins, Fumonisin, Fusaric acid, Moniliformin
<i>Fusarium poae</i>	Aurofusarin, Beauvericin, Butenolide, Culmorin, Cyclonerodiol, Enniatins, Fusarins, Trichothecenes, Diacetoxyscirpenol, Nivalenol
<i>Fusarium proliferatum</i>	Beauvericin, Enniatins, Fumonisin, Fusaproliferin, Fusaric acid, Fusarins, Moniliformin
<i>Fusarium semitectum</i>	Beauvericin, Moniliformin, Trichothecenes, Diacetoxyscirpenol, Zearalenone
<i>Fusarium solani</i>	Fusaric acid
<i>Fusarium sporotrichioides</i>	Aurofusarin, Beauvericin, Butenolide, Culmorin, Enniatins, Fusarins, Moniliformin, Trichothecenes, Diacetoxyscirpenol, HT-2, T-2
<i>Fusarium subglutinans</i>	Fumonisin, Fusaproliferin, Fusaric acid, Moniliformin
<i>Fusarium temperatum</i>	Beauvericin, Fumonisin, Fusaproliferin, Moniliformin
<i>Fusarium thapsinum</i>	Fumonisin, Fusaric acid, Moniliformin
<b><i>Fusarium verticillioides</i></b>	Beauvericin, <b>Fumonisin</b> , Fusaric acid, Fusarins

Source (Logrieco et al., 2002; Mesterházy et al., 2012; Munkvold et al., 2019; Omotayo et al., 2019): Bold- fungi species and mycotoxins of study

*Aspergillus* species infect maize kernels in storage and cause kernel rot and aspergillus ear rot in the field as olive-green powdery molds (Munkvold et al., 2019). The severity of aspergillus ear rot, aspergillus infections, and increased mycotoxin production is associated with kernel damage by insects in the field and storage (Munkvold et al., 2019).

*Penicillium* causes blue-green powdery mold on maize kernels (Munkvold et al., 2019) and *Penicillium* seed rot in wheat (Kim et al., 2012). Every genus member may produce diverse types of toxins (Table 2). The mycotoxigenic fungus is known to generate five principal mycotoxins frequently associated with the contamination of cereals. These mycotoxins include zearalenone, ochratoxin, aflatoxins, fumonisins, and trichothecenes, specifically deoxynivalenol and/or nivalenol. Such occurrences highlight the significant risk posed by these compounds in agricultural products. (Omotayo et al., 2019; Tola and Kebede, 2016; Pinton and Oswald, 2014).

### **2.2.2 Fusarium and Aspergillus mycotoxins: Humans and animals health effects**

The prevalent mycotoxins found in maize and other crops pose significant threats and health effects (Table 3). These mycotoxins negatively affect humans, animals, and economic systems by compromising food and feed safety while also impacting agricultural economies and small-scale industries reliant on crops (Kumar et al., 2021).

The health risks associated with consuming mycotoxin-contaminated foods derived from plants and transferring mycotoxins and their metabolites into animal-derived products such as eggs and meat can range from acute to chronic effects. This impact range depends upon the duration of exposure (Omotayo et al., 2019; Armando et al., 2011; James et al., 2007; Yiannikouris et al., 2003). In livestock, ingesting feeds contaminated with mycotoxins results in acute and chronic health effects associated with reduced productivity (Yiannikouris & Jouany, 2002; Bennett and Klich, 2003). Moreover, human exposure to contaminated air and dust containing these toxins may lead to mycotoxicosis (Niculita-Hirzel, 2016; Jarvis, 2002).

Table 3: Mycotoxin in cereals and other associated crops and health effects on human and animals

Mycotoxin	Fungi Species	Crops Affected	Health effects	
			Human	Animals
<b>Aflatoxins (AFTs)</b>	<i>Aspergillus spp.</i>	Maize, rice, peanuts, cottonseeds, soy, spices and other crops	-Immunosuppression, Acute fatal Toxicosis, Acute Hepatitis, Reye's syndrome, Hepatocellular carcinoma resulting along histopathological signs such as (jaundice, low-grade fever, depression, anorexia, and diarrhea) -Retarded growth and development in Childs (Singh, 2018, Munkvold et al., 2019, Kumar et al., 2020).	<b>Pigs:</b> liver cell damage, Jaundice, and hemorrhage (Munkvold et al., 2019) <b>Poultry:</b> Hepatomegaly, nephritis, immune-suppression genotoxicity, oncogenicity, and overall reduced performance (Imran et al., 2020). <b>Cattle:</b> Hepatitis, gastrointestinal disturbances, reduced reproduction, Drop in milk production, immunosuppression, reduced feed efficiency, and anemia (Munkvold et al., 2019). <b>Horses:</b> anorexia, fever, rapid heart rate, ataxia, colic, icterus, convulsions, bloody feces, and abdominal straining (Munkvold et al., 2019)
Ochratoxins (OTA)	<i>Aspergillus spp.</i> , <i>Penicillium spp.</i>	Oat, maize wheat, and barely	Carcinogenic, genotoxic, immunosuppressive, resulting in induction of upper urinary tract diseases, Balkan endemic nephropathy (BEN) and Chronic interstitial nephropathy (CIN) (Faucet-Marquis et al., 2014; Bui-Klimke and Wu, 2015).	<b>Poultry:</b> Impaired kidney functions/Nephritis, genotoxicity, and immunosuppression (Imran et al., 2020). <b>Cattle:</b> Feed refusal, decreased milk production, and retained fetal membrane (Fink-Gremmels 2008). <b>Dogs and cats:</b> Vomiting, anorexia, increased thirst, polyuria, ataxia, and death. Lesions include nephritis, hemorrhagic enteritis of the large intestine, and necrosis of lymphoid organs (Zain 2011)
Zearalenone (ZEA)	<i>Fusarium spp.</i>	Wheat, maize barley, oats, and rice,	Carcinogenic, hormonal imbalance resulting in impaired fertility and Premature puberty in children (Massart and Saggese 2010, Bennett & Klich 2003; Yang et al. 2018)	<b>Cattle:</b> Decreased milk production, infertility, decreased conception rate (Munkvold et al. 2019). <b>Pigs:</b> Hyperestrogenism, Feminization, reduced libido in males, Pseudopregnancy, vulvovaginitis, inflammation of the mammary gland, atrophy of the ovaries, and prolapse of the vagina or rectum in females (Yiannikouris & Jouany 2002; Bennett & Klich 2003; Munkvold et al., 2019)
<b>Fumonisin (FBs)</b>	<i>Fusarium spp.</i>	Wheat, maize, barley, oats, rice,	Esophageal tumors, abdominal pain, neural tube defects Carcinogenic, hepatotoxic, nephrotoxic, and immunosuppressive (Munkvold et al., 2019; Omotayo et al., 2019).	<b>Pigs:</b> Pulmonary edema (Haschek et al., 2001), <b>Poultry:</b> Liver lesions and other internal organs, Beak enlargement, and brain edema (Zain 2011). <b>Cattle:</b> Reproductive function impairment (Albonico et al. 2016). <b>Horses:</b> Leukoencephalomalacia, brain necrosis, lesions hypersensitivity, and death (Yiannikouris & Jouany 2002). <b>Sheep:</b> Mild liver and kidney inflammation (Munkvold et al. 2019). hepatic cancer in rats death (Yiannikouris & Jouany 2002)
Trichothecenes and their related compounds Mostly <b>Deoxynivalenol (DON)</b> and Nivalenol	<i>Fusarium spp.</i>	Wheat, maize barley, oats, and rice,	Gastroenteritis includes diarrhea, nausea, vomiting, and abdominal pain; Reproductive effects; Toxicosis (Pinton and Oswald, 2014; da Rocha et al., 2014)	<b>Pigs:</b> emesis and reduction in weight gain (Pinton and Oswald, 2014) <b>Cattle:</b> Hematotoxicity, feed refusal, gastrointestinal disturbances, immunosuppression (Imran et al., 2020) <b>Poultry:</b> reduction body weight gain, reduced feed intake, development of ulcers and plaques in the buccal cavity of 7-day-old broiler chicks (Zain 2011)

### **2.2.3 Global distribution of mycotoxin**

The geographical distribution of toxigenic fungi serves as an indicator of their ecological demands. Specifically, thermophilic fungi are predominantly found in tropical latitudes, while psychrophilic fungi are more prevalent in pole latitudes (Van der Fels-Klerx et al., 2016). Notwithstanding the existence of similar diseases and mycotoxins, there is a notable geographical disparity regarding disease severity, the diversity of pathogen species, the toxins commonly encountered, and the consequent management strategies employed in agricultural products globally (Logrieco et al., 2021).

The distribution of these fungi is extensive, encompassing both tropical and temperate zones; however, their influence is particularly significant in tropical and subtropical areas, specifically those located between 40° North and 40° South of the equator (Munkvold et al., 2019; Suleiman and Kurt, 2015). Several factors play a critical role in the observed disparities, including geographical location, environmental conditions, farming practices, and the availability of resources (Logrieco et al., 2021; Adeyeye, 2016).

It is posited that cereals cultivated in temperate climates may harbor substantial levels of *Fusarium* mycotoxins (Munkvold et al., 2019; Tima et al., 2016; Mesterházy et al., 2012), whereas in tropical and subtropical areas, *Aspergillus* mycotoxins are predominant (Mesterházy et al., 2012; Magnoli et al., 2007). These fungi thrive in warmer environments and produce aflatoxins in drought-affected groundnuts and maize fields (Bhat and Miller, 1991). Conversely, *Penicillium* species, particularly ochratoxin A, exhibit higher prevalence in cereals cultivated in colder climates, as evidenced by Lund and Frisvad's (2003) findings in northern Europe. Various climate-related factors interactively influence the colonization of these fungi and the resultant mycotoxin production (Tola and Kebede, 2016; Zain, 2011).

Mycotoxins' specific geographical distribution aligns with each fungal genus's distinctive ecological requirements. Palumbo et al. (2020) indicate that every fungal species has unique environmental requirements favoring its growth and mycotoxin synthesis. For instance, *Aspergillus flavus* thrives under warm, dry conditions (Giorni et al., 2016), while *Fusarium verticillioides* is favorable in warm temperatures with moderate precipitation. *Fusarium graminearum*, on the other hand, exhibits optimal development under mild temperatures

paired with high rainfall during the maturation of maize grain (Bhatnagar et al., 2014). The principal ecological factors affecting fungal colonization on maize grain substrates encompass 1) temperature, 2) relative humidity, 3) rainfall, which affects moisture content, and most critically, 5) water activity ( $a_w$ ) or vapor pressure of water (Giorni et al., 2011; Lazzaro et al., 2012).

#### **2.2.4 Factors for mycotoxin production and contamination**

The production and contamination of mycotoxins in food and feed involve a complex interplay of various factors throughout their production chain (Tola and Kebete, 2016). These factors operate interdependently to influence the colonization of fungi and the subsequent synthesis of mycotoxins. They can be broadly categorized into physical, biological, and chemical factors (Zain 2011).

Physical factors encompass environmental parameters that promote fungal colonization and mycotoxin generation, including temperature, relative humidity, pH, light exposure, and moisture content or precipitation (Kumar et al., 2020; Van der Fels-Klerx et al., 2016). These parameters directly impact fungal infection and growth (Nazari et al., 2014; Medina et al., 2017). Stressors such as drought, rising temperatures, and increased humidity can selectively influence mycotoxigenic fungi's colonization patterns and metabolic processes, thereby affecting mycotoxin production (Medina et al., 2017).

Biological factors pertain to the dynamics between colonizing mycotoxigenic fungi and their substrate, which includes plant species or food sources. Variation in crop hybrids results in differing susceptibility to fungal colonization; however, environmental conditions may exacerbate the vulnerability of even those plant species that are typically resistant (Zain, 2011).

Chemical factors relate to the impact of agricultural inputs such as fertilizers and fungicides (Zain, 2011). Consequently, optimal physical and chemical conditions facilitate colonization by toxigenic fungi in plants and foods (Belli et al., 2007; Milani, 2013; Abramson, 1998). The degree to which specific plant species are vulnerable to colonization by fungi and the subsequent production of mycotoxins is contingent upon their inherent

resistance capabilities, which are influenced by the physical and chemical factors outlined (Virág et al., 2020).

Additionally, interactions with agro-technical practices, such as plant density and irrigation, further modify the roles of these various factors in crop production and their potential for influencing toxic fungal colonization.

### **2.3 Effects of annual meteorological conditions on maize production and mycotoxin contamination**

Climate change represents a substantial alteration in average meteorological phenomena and the variability of weather patterns over the years. This phenomenon is shaped by multiple elements, especially human activities' modifications to the natural environment. These climatic shifts are frequently associated with increasing global temperatures and rising extreme weather events. Notably, changes in precipitation and temperature patterns exert a direct and often detrimental effect on the quantity and quality of agricultural crop yields (Wheeler and Braun, 2013).

Numerous research efforts have explored the potential consequences of anticipated climate changes on food production systems (Parry et al., 2005; Fischer et al., 2005; Lobell and Field, 2007; Vučetić, 2006; Blanc, 2012). According to Lobell and Field (2007), around 30% of the fluctuations in average yields of the three global extensively cultivated cereal crops, maize, wheat, and rice, are attributed to seasonal precipitation and temperature variations. The cultivation of these crops represents over 42% of global cropland (Lobell and Gourdj, 2012) and provides 90% of Global cereals, 42% of calories, and 37% of protein intake (FAO, 2020).

Crop year effects on maize and other crop production lie in the suitability of the agroecological parameters in every respective year. The parameters are associated with stresses that may directly affect the crop's growth and development or create a suitable environment, favoring the development of further stressors like plant pathogens and pests to infest the crop and significantly impact the yield and quality of the harvested produce. Brought by crop year, rainfall, temperature, and relative humidity affect productivity, aside from other factors, including soil fertility and crop nutrient requirements.

### **2.3.1 Effects of rainfall on maize production**

Water availability and heat stress represent critical environmental factors that constrain crop growth, development, and overall yield (Prasad et al., 2008). Fluctuations in water availability, whether interannual or intra-seasonal, predominantly hinder agricultural productivity in numerous regions worldwide; more than 50% and 80% of global agricultural land and maize rely on rainfall, respectively (Aakash et al., 2022). Consequently, analyses concerning the potential threats climate change poses to crop production have primarily concentrated on the implications of water shortages (Harrison et al., 2011).

The relationship between crop yield and the amount of water available to the crops is intricately interconnected (Plavsic et al. 2007). In recent decades, the phenomenon of climate change has led to an increase in weather extremes. From 1860 to 1900, dry and wet years were relatively balanced, each making up 22.5% of the total, while more than half of those years exhibited a standard average pattern, accounting for 55%. However, from 1980 to the 2000s, there was a notable rise in the incidence of dry years, which increased to 52.6% (Sárvári and Pepó., 2014). Deficit and excess water supply to crop requirements are stresses that negatively affect the growth and development of the crop. In a long-term experiment, crop year significantly influences the amount and distribution of rainfall, which impacts maize's growth and yield parameters (Pepó et al., 2009). Favorable crop years yield higher yields, and dry years result in negative yield changes (Bramdeo and Rátonyi., 2020).

The average daily water requirement for maize varies between vegetative and generative phases. In a growing season, optimal maize growth requires around 550 – 670 mm of water aside from the distribution across the phenological stages. The requirement varies from 2mm during vegetative phases to 9 mm in generative phases, particularly during (R0-R3) flowering and early grain-filling stages (Pepó and Sárvári., 2013). Hungarian maize growing season spans from April to September; this marks the critical period for the highest water consumption between mid-June and mid-August, depending on maize hybrid maturity. Years with dry July advocate poor cob development and reduced yield (Nagy., 2007). The amount of rainfall in July demonstrates a strong both-sided relationship with

yield. Hybrid maturity periods, drought tolerance, and adjustment in sowing time are ways to minimize adverse effects (Sah et al., 2020; Dobos and Megyes., 2013).

Imbalances in water availability and distribution during the maize development stage affect its productivity through delays in flowering initiation (Singh et al., 2007), affecting leaf area and reducing the photosynthetic area by reducing interception of photosynthetically active radiation (Bhattacharya and Bhattacharya 2021). Further, an analysis of water supply indicators' influence on yield variability indicated 64% of variability to be associated with precipitation (Žalud et al., 2017).

### **2.3.2 Temperature and maize production**

Temperature variability during crop phenological development plays a significant role in the growth adversely affected by water availability (Kenny and Harrison., 1992). Lobell and Burke (2008) stressed that temperature serves as a critical climatic variable that influences maize production alongside precipitation. However, gradual rises in temperatures during the growing season can lead to significant disturbances in production systems through which ambient temperature affects both the phenological development rates and the physiological functioning of crops (Harrison et al., 2011). Therefore, changes in temperature during crop growth and development are likely to cause a shift in cropping times to minimize the effect.

Harrison et al. (2011) identified that elevated temperatures throughout the growing season may negatively affect agricultural productivity through two primary mechanisms. Firstly, elevated temperatures can hasten the growth rates of certain crops, particularly those whose development is primarily influenced by temperature, such as maize. Shortening plant and grain development time limits the crop's yield potential. Secondly, extreme heat during the flowering period, particularly during the maize silk-tasseling phase, can significantly disrupt pollination processes. This disruption may ultimately lead to the complete inhibition of grain development.

Additionally, in rainfed maize production, temperature increases may speed up plant development, causing the reproductive period, the stage that needs the most water, to shift away from the usual wettest time in the cropping season (Sánchez et al., 2014). The

acceleration of crop development due to increased temperatures reduces plants' capacity to absorb solar radiation at each growth phase. When considering the cumulative effect throughout the entire cultivation period, the decreased interception of solar energy presents significant challenges affecting photosynthesis (Murchie et al., 2009)

The influence of temperature on shortening the duration of the growth cycle, particularly during the grain-filling stage, stands out as a critical factor in elucidating the decline in yields observed at elevated temperatures (White & Reynolds 2003). In Hungary, Bramdeo (2021) reported that maize attains optimum vegetative and generative phase (flowering to yellow ripening) growth spans at 18-20 °C and 24-26 °C, respectively; thus, temperatures above 30 °C during flowering (July), accelerates the decline in pollination and grain filling. This is further reported by Pepó and Csajbók (2013), who noted a strong positive and negative correlation between July rainfall and temperature, respectively.

The yield of maize is determined by two primary factors: the quantity of grains (kernel number) produced and the weight of kernels (Harrison et al., 2011). The study further reports that optimal kernel weight is realized when the rate of grain filling and the total duration of this filling process are maximized. The peak rate of grain filling is obtained in moderately elevated temperatures, ranging from 25 to 32 °C. Nevertheless, these elevated temperatures also expedite the plant's overall developmental process, resulting in a reduced duration for the grain filling period. Shim et al. (2017) indicate that increased temperatures lead to a decline in yield, primarily due to a reduction in kernel number rather than kernel weight. This finding is supported by Lizaso et al. (2018), who discuss the impact of heat stress on the viability of pollen, which subsequently influences the number of kernels produced. The effect of elevated temperature on the generative phase and yield and its influence on soil water status implies that a reduction in rainfall coupled with temperature elevation leads to negative impacts on maize yield. High temperature or insufficient moisture during the silk-tasseling phase of maize, which encompasses flowering and pollination, leads to a more significant decrease in crop yields than other climatic stresses (Harrison et al., 2011). Elevated temperatures and dry conditions accelerate the release of pollen while simultaneously delaying the emergence of silks, thus reducing the overlap period between these critical reproductive stages (Wang et al., 2021; Harrison et al., 2011).

Furthermore, research indicates that the germination capacity of pollen on silks is significantly reduced when temperatures exceed 32°C (Basra, 2000) and sharply reduced at 38 °C (Wang et al., 2021). Further, elevated temperatures exceeding 38°C prolong the silk emergence duration and lessen the silk emergence ratio, ultimately leading to a significant decrease in seed set (Liu et al. 2022).

In an analysis by Lobell and Field (2007), warming trends were identified as significant factors contributing to the reduction of global agricultural productivity over decades. However, the analysis indicates that enhancements in farming technologies and potential benefits from CO<sub>2</sub> fertilization have increased overall crop yields. These gains are offset by the responses of plants to elevated temperature levels. This negative impact is expected to intensify due to rising mean temperatures and more significant variability within climatic conditions (Porter and Semenov 2005, Lobell and Field 2007). Particularly severe consequences are projected for regions located at lower latitudes, where many cereal-producing areas are already close to critical temperature thresholds for plants (Easterling et al., 2007). It is crucial to comprehend historical temperature trends within the framework of their implications for crop yields, as understanding these dynamics plays a vital role in assessing the effects of regional climate changes on agricultural production.

### **2.3.3 Relative humidity and Maize production.**

Relative humidity (RH) presents a dual nature in its impact on crop yield, encompassing both beneficial and negative aspects (Dong et al., 2024). The effects of RH on crop production are aggravated by its influence on air temperature and water/moisture availability. Aylor (2004); Fonseca and Westgate (2005) reported that elevated relative humidity (RH) can prolong the viability of pollen by decreasing moisture loss from pollen grains. Furthermore, elevated relative humidity (RH) has been noted to enhance the process of flower opening during the late afternoon or nighttime. This behavioral adaptation appears to be a strategy employed to mitigate the heat stress that plants typically encounter in the early to mid-afternoon ( van Doorn and van Meeteren (2003). However, on the contrary, studies report that high RH may counteract pollen release by hindering anther opening (Pacini and Dolferus, 2019). This inhibition is anticipated to decrease pollen shed quantity and seed set under heat stress conditions. Dong et al. (2024) conclude that, despite the

potentially detrimental effects, elevated RH yields significantly more favorable outcomes for seed sets and mitigates the adverse effects of heat stress on maize yield during the flowering period.

Further, Wang et al. (2021) reported that under high temperatures initial emergence of silk is limited. Dong et al. (2024) revealed a negative correlation between the silk emergence ratio on high temperature and vapor pressure deficit (VPD) while demonstrating a positive correlation with relative humidity (RH), suggesting that increased RH mitigates the detrimental effects of high temperature on silk elongation. Silk is vital for seed sets in maize and is sensitive to water stress as it presents tissues with the highest water content (Nielsen, 2016). Under prolonged heat stress, lower RH reduces plant growth and the activities of photosystems I and II (Lysenko et al., 2023).

Therefore, high relative humidity reduces water loss in plant systems and sustains optimal leaf productivity. This effect, in turn, enhances the ratio of silk emergence, particularly under temperature-stress conditions (Dong et al., 2024). Additionally, the development of silk is markedly influenced by soil moisture levels (Westgate and Boyer 1985). The elongation ratio of silk appears to correlate closely with leaf water potential, which tends to align with the silk's water potential (Westgate and Boyer 1986). High VPD increases water loss from the soil, resulting in dry and heated soil (drought) and incomplete silk emergence (Oury et al., 2016). High RH can further leverage this by reducing water loss from the soil and minimizing soil water deficiency.

#### **2.3.4 Annual meteorological conditions and mycotoxins contamination of maize**

The occurrence of mycotoxin-producing fungi in maize is closely linked to annual meteorological conditions, reflecting trends in weather patterns observed over decades (Battilani et al. 2008). Weather variables, particularly precipitation/water availability, air temperature, and relative humidity, exert significant effects on the growth of toxigenic fungi in staple crops, including maize, wheat, and rice, and consequently influence mycotoxin production (Perrone et al., 2020; Sanchis and Magan, 2004; Van der Fels-Klerx et al. 2013). *Fusarium*, *Aspergillus*, and *Penicillium* are frequently identified as maize-contaminating fungi. In addition to meteorological factors, the prevalence of mycotoxins in maize is dependent upon various elements, which include agronomic considerations (such as hybrid

type, soil characteristics, tillage practices, and preceding crops), storage conditions (involving temperature, humidity, handling procedures, and the presence of pests like insects, rodents, and birds), and the duration of storage (Hell et al. 2000).

In tropical regions, the prevailing climatic conditions are conducive to the proliferation of fungi, which can lead to significant crop contamination (Perrone et al., 2020). However, the effects of climate change are likely to exacerbate the issue in temperate regions (Herreira, 2023). The occurrence of dry spells during critical phases of plant development may elevate the risk of grain contamination in agricultural fields, particularly by xerophilic and moderately xerophilic fungi such as *Aspergillus* and *Penicillium* (Perrone et al., 2020). Temperature and water availability are critical weather conditions determining *Fusarium* and *Aspergillus* fungi's growth and mycotoxin production (Sanchis, 2004).

In warm, humid subtropical and tropical conditions, *A. flavus/parasiticus* species thrive, causing aflatoxin contamination. In the context of abiotic influences, it has been established that temperature and water availability, measured explicitly as water activity ( $a_w$ ), along with their interplay, are fundamental determinants regulating fungal growth and mycotoxin synthesis (Medina et al., 2017). Sanchis., (2004) investigated the influence of water availability (water activity) and temperature factors on germination, growth, and aflatoxin synthesis by *A. flavus* and *A. parasiticus* in vitro media. The finding indicated that germination can occur across a broader spectrum than growth, with aflatoxin production occurring within an even more restricted range than growth. The ideal conditions for aflatoxin production for both species are identified as 33 °C and water activity of 0.99 (30-32%) moisture content, whereas the optimal parameters for growth are established at 35 °C and an  $a_w$  of 0.95 (26-27%) moisture content.

Warm and comparatively dry conditions at the onset of the growing season can promote the proliferation of *Fusarium* foot rot. This phenomenon facilitates the accumulation of pathogenic material at the bases of plant stems (Meekes and Kohl, 2002). The moisture content at anthesis is crucial in determining the incidence of fungal ear infections. Lacey et al. (1999) established a direct correlation between elevated infection rates during anthesis and prevailing wet weather conditions. In another study, Hope and Magan (2003) conducted in vitro experiments to analyze the production of deoxynivalenol (DON) and nivalenol

(NIV) by a strain of *Fusarium culmorum*, focusing on the effects of water availability quantified as water activity and temperature. Their findings indicated that the environmental parameters conducive to toxin synthesis were more restricted than those that allowed fungal proliferation. Notably, toxin formation was observed exclusively under relatively high moisture conditions that facilitated rapid growth of the fungi. The optimal moisture range for the simultaneous production of both DON and NIV was identified as 25–30%, aligning with moisture levels

typically found in harvested grain during years with elevated rainfall and wet conditions. Furthermore, the ideal temperature for mycotoxin production was 25 °C. This research underscores the substantial risk of contamination during warm, wet years, providing valuable insights for predicting periods of heightened mycotoxin risk based on weather monitoring.

Since mycotoxins-producing fungi comprise endophytic and epiphytic microflora components found in main food crops, they are naturally occurring compounds that cannot be avoided in the field. Crops like corn and mycotoxin-producing fungi like *A. flavus* may change their geographical location and distribution due to climate change and global warming (Perrone *et al.*, 2020). This would make it more likely that produced mycotoxins are present in other locations. *A. flavus* infection of crops and the formation of aflatoxin in the field may also be facilitated in certain regions by climate change and drought conditions (Wu *et al.*, 2011). By facilitating fungal colonization, elevated CO<sub>2</sub> levels are likely to enhance further mycotoxin production in crops infected with *Aspergillus* and *Fusarium* species (Moretti *et al.*, 2019)

Generally, annual weather variability caused by climate change has demonstrated a significant shift in geographical location distribution of mycotoxigenic-producing fungi, which in turn has resulted in aggravated mycotoxin production and contamination and hence threatening food safety at local, national, regional, international, and global levels as well as global food trade. Climate modeling studies on mycotoxin production and the anticipated impacts might be taken as guides for formulating integrated approaches in managing mycotoxin to reduce the expected impacts. Integrating genetically tolerant crops and agrotechnical factors would bring sustainability and stable, safe food systems.

## **2.4 Maize fertilization and mycotoxin contamination**

Soil fertility influences maize plants' susceptibility to fungal colonization and mycotoxin contamination dynamically depending on other factors, mainly nitrogen (N) fertilization (Bruns et al., 2003). N plays a critical role in the growth and development of plants; however, its available level can adversely influence various attributes crucial for crop yield and quality. The literature presents conflicting insights into how N fertilizer impacts the quality and safety of maize grain, specifically on addressing fungal infestation and regulating mycotoxin contamination (Scarpino et al., 2022)

N deficiencies are primarily associated with increased FB contamination. At the same time, excessive applications of N fertilizer (exceeding 300 kg ha<sup>-1</sup>) have been linked to elevated levels of zearalenone (ZEA) (Blandino et al., 2008; Souza et al., 2016). On the other hand, studies by Marocco et al. (2008), Ariño et al. (2009), and Bocianowski et al. (2020) found that N fertilization tends to exacerbate FB levels in maize while also promoting infection by the pathogen *Fusarium verticillioides*. Similarly, excessive N levels increase FBs levels (Hasegawa et al. 2008; Souza et al. 2016).

Research examining varying nitrogen rates reveals that lower rates effectively reduced fumonisins (FBs), ochratoxin A, and aflatoxins compared to unfertilized control groups. Conversely, increased N fertilization correlated with heightened fungal ear rot severity and increased levels of deoxynivalenol, fumonisins, zearalenone, and ochratoxin A (Blandino et al. 2008). Notably, the incidence of gibberella ear rot diminished at moderate N application rates of 100 kg ha<sup>-1</sup>, while it intensified at higher N rates of 200 kg ha<sup>-1</sup>. Overall, the levels of deoxynivalenol tended to decrease with increased N rate (Reid et al., 2001). This intricate relationship underscores the necessity for more detailed research into the specific impacts of nitrogen management on maize grain quality. Optimal recommendations to mitigate mycotoxin risks suggest maintaining N levels in soil at neither deficient nor excessive thresholds, ensuring sufficient nutrient availability through careful N fertilization practices (Wilson et al., 2005). It is also essential to consider that nutrient deficits and over-fertilization, particularly nitrogen, can increase vulnerability to insect pests and diseases (Tubajika et al., 1999).

## **2.5 Hybrid resistance to toxigenic fungi**

Developing crop hybrids that exhibit resistance to fungal infections is critical for effective mycotoxin management. While no hybrids show complete resistance to toxigenic fungi or mycotoxin production, resistant maize hybrids generally yield lower toxin levels; resistance mechanisms differ based on the disease entry site (Eli, 2022). Specific resistance mechanisms in maize hybrids have been noted concerning ear rots, with resistance linked to kernel and silk channel infections (Mesterházy et al., 2020, 2012; Miller et al., 2003). Therefore, screening of maize hybrids for fungal resistance depends on the infection pathway, such as silk resistance or kernel resistance. Mesterházy et al. (2012) emphasize the necessity of conducting resistance screening for hybrids against toxigenic fungi under field conditions, as greenhouse assays have failed to cause satisfactory infection levels and genotype differentiation.

Schaafsma et al. (1997) report that, in analyzing the conditions for hybrid screening under field conditions, it is evident that artificial inoculation combined with misting is essential due to the annual variability in disease severity associated with natural infections. Kernel wound inoculation and silk channel denote methods employed for inoculation, with several techniques specifically developed for both approaches (Eli, 2022). Evaluating silk and kernel resistance involves injecting a conidial suspension into the silk channel or directly into kernels for kernel resistance (Mesterházy et al., 2020). Moreover, the timing of the inoculation plays a crucial role in ensuring adequate disease severity, which must be balanced to avoid conditions that are lowest or highest extremes, which would hinder the ability to distinguish differences among genotypes (Mesterházy et al., 2012). The onset of the silk browning stage indicates the best time for silk channel inoculation (Schaafsma et al., 1997), while kernel inoculation should occur 6 days after 50% mid-silking (Mesterházy et al., 2022). Further, Mesterházy et al. (2012) indicate that, regardless of the method employed, researchers are advised to identify the primary mode of entry of *Fusarium* spp that is of interest in the geographical location attributable to an often weak correlation between kernel and silk channel infections, with hybrids showing varied resistances.

### 2.5.1 Hybrid resistance to Fusarium ear rot and toxin production

Fusarium spp. ear infection results in giberella ear rot (GER) and Fusarium ear rot (FER). GER is mainly caused by *F. graminearum* and *F. culmorum*, while *F. verticillioides* and *F. proliferatum* mainly cause FER. There are ear rots in maize that naturally occur. Mesterházy et al., 2012 reported the most significant maize ear rot epidemic in two decades in Hungary, with disease severity levels reaching 35% across eight sites where the highest-yielding hybrids from various leading companies were evaluated. The hybrid responses varied, with reactions between 27% and 48%. Additionally, the overall severity of ear rot, measured as coverage, was calculated at 10.2%, ranging from 7% to 14%. Notably, the site with the highest infection levels exhibited an average disease incidence of 63%, ranging from 28% to 87%, and a mean severity of 20%, with values between 8% and 29%.

GER genetic variability exists in maize hybrids (Lanubile et al., 2014; Mesterházy et al., 2012). To fully leverage the potential of this genetic variability, a comprehensive understanding of the genetic structure underlying the trait is essential (Kebede et al., 2016). Most maize varieties utilized in commercial agriculture are hybrids, necessitating the creation of inbred lines to introduce the resistance of giberella ear rot (GER) into the hybrid breeding process (Mesterházy et al., 2012). Kebede et al. (2016) conducted an assessment of 410 recombinant inbred lines (RILs) to determine their resistance to gray ear rot (GER) across a span of three years. This evaluation employed both silk channel and kernel inoculation methodologies. In addition to the resistance evaluation, the RILs were analyzed for various agronomic characteristics, including the duration until silking, the extent of husk coverage, and the rate of kernel drydown. The RILs exhibited notable genotypic differences across traits, with high heritability estimates. Weak but significant correlations ( $P < 0.01$ ) were found between disease severity and agronomic traits, indicating their relevance to disease resistance. Common QTLs were identified for GER resistance and kernel drydown rate, hinting at pleiotropic genes that could enhance both traits simultaneously.

Using composite interval mapping, Ali et al. (2005) identified 11 QTLs related to resistance against Gibberella ear rot after silk inoculation and 18 QTLs following kernel inoculation across four different environments. Notably, only 2 QTLs on linkage group 7 were observed consistently across multiple tests for silk resistance, and 1 QTL on linkage group 5 was

repeatedly detected for kernel resistance. These loci accounted for a proportion of the total phenotypic variation ranging from 6.7% to 35%. This observation underscores the significant impact of environmental factors on the expression of these traits. Thus, germplasm and the markers associated with the QTLs demonstrating substantial phenotypic effects have the potential to be valuable in marker-assisted selection processes aimed at integrating *Gibberella* ear rot resistance into commercial corn varieties.

Therefore, mechanisms for GER resistance seem complex and can be influenced by several factors (Eli, 2022). They include environment (Ali et al., 2005), several QTLs (Kebede et al., 2016; Ali et al., 2005), silk and kernel characteristics (Kebede et al., 2016), and agronomic operations (Munkvold, 2003). The intricacies account for the increased variability in the resistance of hybrids over years and locations (Mesterházy et al., 2022). Dalla Lana et al. (2021) evaluated 15-16 hybrids in 10 locations for 4 years, focusing on GER and DON contamination. They found year-to-year variations in DON but consistent hybrid rankings for mycotoxin levels. GER-resistant genotypes displayed greater stability, indicating that selecting less susceptible hybrids could efficiently manage GER.

### **2.5.2 Hybrid resistance to *Aspergillus* ear rot and toxin production**

The genus *Aspergillus* encompasses numerous species that exhibit a global distribution, primarily inhabiting soil and various crops, often in maize and other plant products (Lević et al., 2013). *Aspergillus flavus* is the predominant species implicated in *Aspergillus* ear rot. Although *A. flavus* functions as a saprophyte, it can induce considerable corn ear rots and kernels, given conducive environmental conditions, both pre-harvest in the field and post-harvest during storage phases. This species can produce aflatoxins, which are recognized as some of the most toxic naturally occurring compounds (Warburton et al., 2013). Implementing resistant maize hybrid cultivation emerges as the most proficient strategy to mitigate the incidence of *Aspergillus* ear rot and reduce aflatoxin levels.

*A. flavus* is a xerophilic fungal species that exhibits physiological adaptations enabling its survival in stressful environmental conditions (Magan., 2007). Elevated average temperatures and prolonged drought periods induce heat stress in crops, favoring aflatoxin contamination (Abbas et al., 2002; Moretti et al., 2004). Additionally, insects play a critical role in the proliferation of *A. flavus* on corn grains by acting as vectors for conidia

transmission. These pests not only facilitate the spread of the fungus but also inflict mechanical damage on the grains, thereby allowing the pathogen to infiltrate the unprotected endosperm (Lević et al., 2013; Battilani et al., 2011). A range of preventive measures has been recommended to reduce the occurrence of aflatoxin in agricultural environments. These include selecting maize hybrids with enhanced resistance to both abiotic and biotic stresses, adhering to optimal sowing schedules, implementing crop rotation practices, ensuring proper plant nutrition and irrigation, as well as controlling insect populations, diseases, and weeds (Bruns, 2003; Widstrom et al., 2003)

Consequently, several research are devoted to identifying novel sources of resistance (Munkvold, 2003; Daves et al., 2010; Cary et al., 2011; Warburton et al., 2015; Wahl., et al., 2017; and Windham et al., 2018). Several methodologies are employed in the development of hybrids that are resistant to aflatoxin, which encompasses molecular techniques (Mideros et al., 2014), studies on antifungal proteins (Chen et al., 2015), and analyses of the morphological traits of ears and grains in resistant hybrids (Brown et al., 2001). Given the correlation between *A. flavus* infection and drought stress, one strategic approach involves the development of drought-resistant hybrids (Abbas et al., 2012; Farfan et al., 2012). Several studies have also examined the potential for insect-resistant hybrids to indirectly minimize aflatoxin synthesis (Williams et al., 2005; Abbas et al., 2006).

Furthermore, various genetic factors are crucial in determining the level of maize resistance against *A. flavus* infection and the subsequent accumulation of aflatoxins. These factors include husk tightness, which helps shield the maize kernel from fungal invasion, and resistance at the pericarp and subpericarp levels (Cary et al., 2011). Further, the maize plant can produce specific proteins that are directly associated with inhibiting *A. flavus* growth. These proteins include a germination-induced ribosome-inactivating protein (RIP) and zeamatin (Guo et al., 1998). Other relevant proteins include chitinase (Huang et al., 1997) and trypsin inhibitor protein (TI) (Moore et al., 2004; Chen et al., 2004). Furthermore, the structural integrity provided by wax and cutin layers in maize kernels (Gembah et al., 2001; Guo et al., 1995) contributes substantially to the plant's natural defenses against *A. flavus* and aflatoxin production, enhancing overall maize resilience. The primary challenge in developing maize hybrids resistant to *A. flavus* lies in the interplay between genotype and environmental factors (Fountain et al., 2014). Thus, it is imperative to identify maize

genotypes demonstrating stability in resistance across diverse environmental conditions (Warburton and Williams, 2014). Enhancements in artificial inoculation methods could be one of the most effective strategies for accurately identifying maize genotypes resistant to *Aspergillus* ear rot and the associated aflatoxin production. Additionally, considering nitrogen requirements as a critical limiting agronomic variable in maize yield, along with its impact on the colonization of fungal pathogens and subsequent mycotoxin generation, this research study aims to assess the stability of different resistance sensitivities in selected maize hybrids under varying nitrogen conditions across varying production years to address the challenge.

### **3. MATERIALS AND METHODS**

#### **3.1 Experimental site**

The three-year study commenced in the spring seasons of 2022, 2023, and 2024 at the crop production experimental station Látókép of Debrecen University, Hajdú-Bihar, Hungary. This site is geographically positioned at 47°33'42" N latitude and 21°27'02" E longitude, approximately 15 kilometers from the city of Debrecen. The experiment station was set up in 1983, initially to conduct experiments on fertilization and crop rotation. The site's soil characteristics are defined by a uniform layer of calciferous chernozem formed on the loess ridge of Hajdúság. The top layer reveals an average humus content ranging from 2.7% to 2.8%, with a depth of roughly 0.8 meters. The acidity levels in the upper soil strata are near neutral, indicated by a  $\text{pH}_{\text{KCl}}$  range of 6.46 to 6.6. The phosphorus availability in this calcareous soil is average, with AL-soluble  $\text{P}_2\text{O}_5$  measured at  $133 \text{ mg kg}^{-1}$ . The soil supply of potassium falls into the average-good category, with AL-soluble  $\text{K}_2\text{O}$  recorded at  $240 \text{ mg kg}^{-1}$ . The soil's plasticity index ( $\text{K}_A$ ) also varies between 43 and 47.6. Further, wheat served as the forecrop at the experimental site.

In all growing seasons, the moisture content of the soil at the time of sowing was conducive to the germination of seeds. This favorable moisture condition was sustained throughout the entire growing period. However, the 2022 growing season experienced dryness in May, June, and July, necessitating two supplementary irrigation events. Two significant interventions were carried out. One in May and the other in early July during crucial physiological phases of plant development. These phases include the vegetative stage, flowering stage (encompassing tasseling and silking), and the grain filling period. Figure 2 visually represents the climatic and weather conditions experienced during the experimental crop years, compared to the average conditions documented over the previous three decades.

#### **3.2 Agrotechnical operations during the experiment years**

During the experimental years, the agrotechnical operations (table 4) included tillage operations, fertilizers applications, sowing, weed management, irrigation (2022), and harvesting.

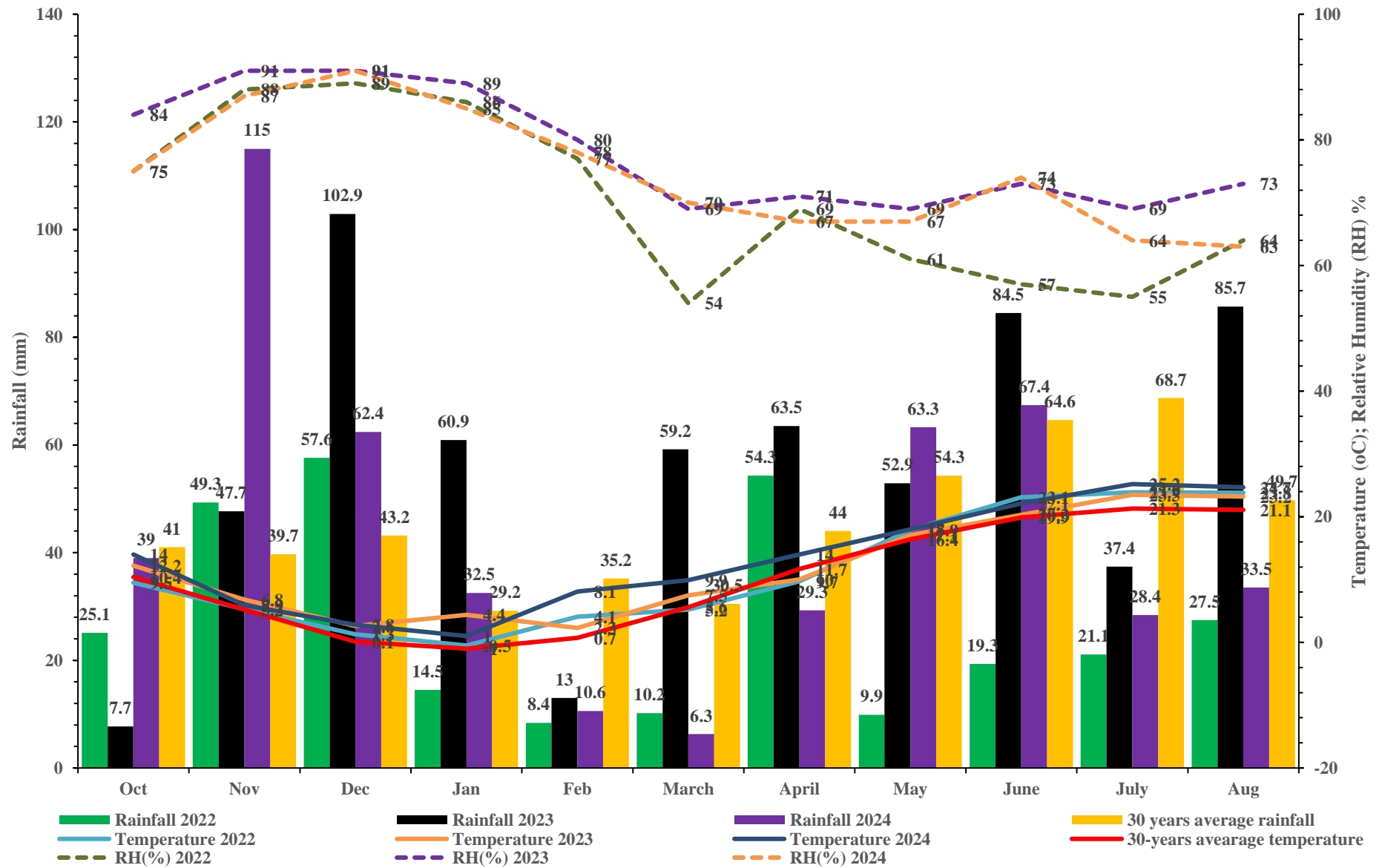


Figure 2: Meteorological data of the Látókép research station during the experiment years and the average for the past 30 years (Debrecen: 2022-2024).

Table 4: Agrotechnical operations in the experimental years, Latókép – Debrecen (2022-2024)

<b>2022/2022</b>	<b>2022/2023</b>	<b>2023/2024</b>
<b>Forecrop winter wheat</b>	<b>Forecrop winter wheat</b>	<b>Forecrop winter wheat</b>
08 August 2021 – discing+rolling	25 July 2022 – discing+rolling	25 July 2023 – discing+rolling
25 August 2021- discing+rolling	15 August 2022 – fertilization N 50%+P	28 July 2023 – fertilization N 50%+P and K
25 August 2021 – fertilization N 50%+P and K 100%	and K 100%	100%
25 July 2021 – discing+rolling	15 August 2022 – discing+rolling	28 July 2023 – discing+rolling
15 September 2021 – ploughing	10 October 2022 – ploughing	07 October 2023 – discing+rolling
02 March 2022 – combinator	03 March 2023 – combinator	10 October 2023 – ploughing
28 March 2022 - fertilization N 50%+ combinator	14 April 2023 - fertilization N 50%+ combinator	20 March 2024 – soil leveling and closing
12 April 2022 – planting 72 000 seeds/ha	24 April 2023 – planting 72 000 seeds/ha	05 April 2024 - fertilization N 50%+ combinator
12 May 2022 – herbicid Laudis 2,0 l/ha	25 May 2023- row cultivator	09 April 2024 – planting 72 000 seeds/ha
16 May 2022 – row cultivator	30 May 2023 – herbicid Laudis 2,0 l/ha	09 May 2024 – row cultivator
31 May 2022- row cultivator	31 May 2023- row cultivator	15 May 2024 – herbicid Laudis 2,0 l/ha
14-15 June 2022- irrigation 30mm	20 September 2023 - harvest	29 May 2024 – row cultivator
02-03 July 2022- irrigation 30mm		04 September 2024 - harvest
03 October 2022 - harvest		

### 3.3 Experimental design and treatment combinations

The experiment was carried out in four replications using a split-split plot design. The study tested three (3) maize hybrids selected from registered companies in Hungary. The yield and mycotoxin contamination of DKC4590 (FAO 360), GKT376 (FAO 360), and P9610 (FAO 340) denoting the 3 maize hybrids used were investigated on different nitrogen treatments and fungi inocula isolates infections. The companies and hybrids included Pioneer (P9610) with undefined sensitivity and high yielding, Bayer (DKC4590) with a defined high tolerance level, and the Cereal Research Nonprofit Ltd-GK Szeged (GKT376) with a low tolerance level. The main plot factor was the maize hybrids, the sub-plot treatment was N fertilizer dosage (0, 90, and 150 kg ha<sup>-1</sup>), and the sub-subplot intervention involved the application of fungal ear inoculation. The treatment combinations are presented in Figure 3 below.

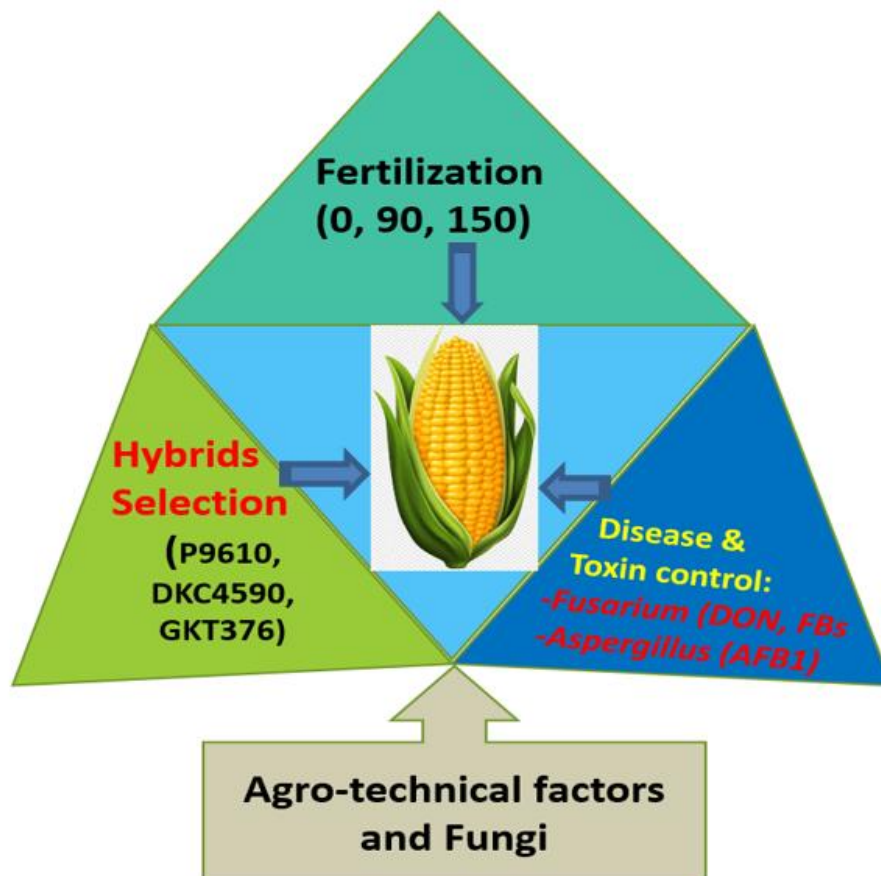


Figure 3: Agrotechnical treatment combinations

The dimensions of each subplot were 5 m by 3.04 m, resulting in a total area of 15 m<sup>2</sup> and 0.76 m row spacing, making four rows plot<sup>-1</sup>, each containing roughly 25 plants. A 1 m

space marked the distance between adjacent blocks. In this design, three rows were subjected to artificial inoculation using specified fungal isolates, adhering to established international protocols stipulating that one inoculum should be utilized for each distinct pathogen. Specifically, the first row was inoculated with a strain of *Fusarium graminearum*, known for its deoxynivalenol (DON) production. The second row received a strain of *Fusarium verticillioides*, responsible for producing fumonisins (FBs). The third row was inoculated with a strain of *Aspergillus flavus*, recognized for its production of aflatoxins (AFBs), specifically measuring AFB1. Lastly, the fourth row served as the untreated control.

### 3.4 Fungal isolate collection and inoculation procedures

The isolates of *Fusarium graminearum*, *Fusarium verticillioides*, and *Aspergillus flavus* employed researched were sourced from Cereal Research Nonprofit Ltd, Szeged, Hungary, and are part of the collection of micro-organisms from naturally infected grain in Hungary. The inocula was prepared per the guidelines of Szabo et al., (2018). Subsequently, inoculation was carried out using the tooth-pick method established by Young (1943), as modified by Mesterházy (2008), to assess kernel resistance (Figure 4).

In all experimental years, inoculation was done 6 days after reaching 50 percent mid-silking, and inoculum tooth-picks were inserted into the center of the upper ear. It was accomplished by creating a hole with an awl measuring 15 mm long and 1.5 mm in diameter. The toothpicks were left in place until the time of harvest.



Figure 4: toothpick inoculation procedures: (1) Inoculum toothpicks, (2) Making a hole with an awl, (3) Hole on the ear, (4) Inoculum toothpick inserted

### 3.5 Data collection procedures

#### 3.5.1 Evaluation of ear and kernel rot severity

In order to enhance the sampling accuracy, the assessment focused exclusively on ears that exhibited toothpick marks, using 15 cobs plot<sup>-1</sup> for every fungus strain (Figure 5). Upon reaching maturity, with a permissible harvest moisture content of nearly 20%, the cobs were manually harvested and dehusked to assess the extent of kernel and ear rot. The evaluation of fungus-induced ear rot severity was quantified using a percentage scale (Mesterházy et al., 2022). The incidence expresses ear coverage percentage on an average regular ear, which contains 700-800 grains. Thus, an infection of 1% is established when the ear displays 7 to 8 kernels with observable signs of infection. At the same time, a 0.15% infection rate is recognized when just a single kernel shows visible signs of infection. The severity assessment incorporated two data categories: one indicating direct infection from the tooth-pick method and the record from naturally occurring infection independent of this method.



Figure 5: Ears inoculated with fungal isolates of FG, AF, and FV vs untreated controls

### **3.5.2 Sample preparations and measurement of mycotoxins**

In mycotoxin analysis, five cobs exhibiting a moderate occurrence of ear rot, devoid of any insect damage, were systematically chosen from each row. These selected ears were placed in a mesh-lined Rashel bag and subjected to dry storage for two weeks. Following this period, the ears were shelled. The resultant grains underwent a coarse grinding process, followed by thorough mixing, before being finely milled to facilitate the measurement of toxins.

Mycotoxin amounts were measured using an enzyme-linked immunosorbent assay (ELISA) utilizing ELISA kits produced by Romer Labs, Tulln, Austria. The quantification of FBs deoxynivalenol and aflatoxin B1 was performed accordingly by;

- i) Agra-Quant FBs 0.25/5.0 ELISA kit,*
- ii) Agra-Quant DON 0.25/5.0 ELISA kit and,*
- iii) Agra-Quant AFB1 2/50*

A direct competitive assay evaluated the samples at a wavelength of 450 nm utilizing a microtiter plate reader: Thermo Scientific, Waltham, Massachusetts, USA. Each measurement was carried out in quadruplicate, with a coefficient of variation (CV%) < 15%. The detection thresholds established by the ELISA kit were determined to be 0.2 (ppm) for Deoxynivalenol (DON) and total fumonisins (FBs), while Aflatoxin B1 (AFB1) exhibited a detection limit of 2 (ppb).

### **3.5.3 Measurement of physiological and quality parameters**

Measurement of physiological parameters namely relative chlorophyll content (SPAD index), Leaf area index (LAI), and the canopy reflectance parameter of normalized differences vegetation index (NDVI). The measurement was done in several growth stages of the crop during the vegetative phase and reproductive/generative phases according to the IOWA State University plant growth scale.

#### **3.5.3.1 Relative chlorophyll content (SPAD index)**

The leaves' relative chlorophyll concentration and nitrogen status were measured with a handheld chlorophyll meter (Minolta SPAD-502 plus). The ten youngest fully expanded

leaves were randomly chosen at each test unit for measurement using the double mid rows of each plot. The non-destructive measurement began 50 days after sowing and continued throughout the growth season. The SPAD meter calculates unit values for leaf chlorophyll concentration as described by Wood et al. (1993) in the equation (i);

$$SPAD\ Index = A \times \left[ \log \left( \frac{I_{or}}{I_r} \right) - \log \left( \frac{I_{of}}{I_f} \right) + B \right] \dots\dots\dots(i)$$

Where:

- A; B = constants;*
- I<sub>or</sub> = current from red detectors with a sample in place;*
- I<sub>r</sub> = current from red infrared detectors with sample in place;*
- I<sub>of</sub> = currents from red detectors with no sample and,*
- I<sub>f</sub> = currents from infrared detectors with no sample*

**3.5.3.2 Leaf area index (LAI)**

The Leaf area index (LAI) was assessed using the Delta-T SunScan SS1 COM-R4 portable plant canopy analyzer system, equipped with a radio link, developed by Delta-T Devices Ltd., UK. This system measures light transmission and examines the number of incidents and photosynthetically active radiation (PAR) transmitted within crop canopies. The probe, which is 100 cm long, contains 64 PAR sensors with a spectral range of 400–700 nm. The data is presented in units of PAR quantum flux (µmol m<sup>-2</sup> s<sup>-1</sup>) and LAI units (m<sup>2</sup> m<sup>-2</sup>).

**3.5.3.3 The normalized difference vegetation index (NDVI)**

NDVI was assessed during various stages of development using the "Trimble Greenseeker" NDVI meter. According to Panek et al. (2020), this instrument calculates NDVI through the analysis of red light (650 ± 10 nm) and near-infrared light (770 ± 15 nm), as described in equation (ii) below,

$$NDVI = (NIR - RED) / (NIR + RED) \dots\dots\dots(ii)$$

Readings were taken for the plot with the equipment positioned 1.0 m above the maize canopy.

**3.5.3.4 Harvesting and grain yield**

The grain yield in a 7.6 m<sup>2</sup> plot was measured and converted into kg ha<sup>-1</sup> by a two-row Sampo Rosenlew SR 2010 plot combine harvester (Figure 6) using a Coleman weighing system.



Figure 6: Harvesting- Sampo Rosenlew SR 2010 plot combine harvester

The grain yield was adjusted to 14% moisture content following Badu-Apraku et al. (2012), as shown in equation (iii),

$$\text{Grain yield (kg ha}^{-1}\text{)} = \text{Grain yield} \times (100 - \% \text{ AMC}) / (100 - \% \text{ SMC}) \times 100 \dots\dots\dots(iii)$$

Where:

AMC = Actual (obtained) Grain Moisture Content (%),

SMC = Standard Moisture Content

### 3.5.3.5 Protein, moisture content, and starch

The Pfeuffer Granolyser NIR machine (Pfeuffer, Germany) was used to analyze the grains' protein, starch, and moisture levels. This machine uses NIR diode technology, conducting 1500 individual scans for each sample. The built-in spectrometer scans the sample seeds within the 950 to 1540 nm range.

### **3.6 Statistical procedures for analysis of data**

The impact of nitrogen dosage and maize hybrids on the extent of ear and kernel coverage, as well as the levels of mycotoxin contamination, was assessed through analysis of variance following a normality assessment conducted with the Shapiro-Wilk test. The comparison of treatment means utilized the least significant difference (LSD) and was deemed significant at  $p < 0.05$ . Furthermore, Duncan's multiple range test (DMRT) was meticulously applied to comprehensively compare the various interaction means, as illustrated in the accompanying graphs and detailed tables. The statistical software Genstat 18th edition, registered for Plant Research International, was used.

Regression and Pearson correlation analyses (two-tailed) were carried out to investigate the relationships between the severity and toxins production within and between toxigenic fungal species. It additionally examined the relationship between physiological parameters vs. fungal diseases and mycotoxin production. Further, multivariate analysis using principal component analysis (PCA) was performed using the R program to investigate the intricate interrelationships among various variables. This technique also aimed to decrease the dimensionality of the dataset while preserving essential information. PCA facilitated the identification of patterns and correlations within the examined factors and variables.

## **4. RESULTS AND DISCUSSION**

The results and discussion section provides a detailed analysis of the effects of hybrid varieties and nitrogen fertilization on maize production, as well as their subsequent impact on the colonization and progression of fusarium and aspergillus fungal ear rot diseases and the resultant mycotoxin generation. Additionally, the analysis delves into how variability in weather conditions during the growing seasons contributes significantly to the central themes discussed. Moreover, the understanding and implications of physiological parameters such as relative chlorophyll content and canopy reflectance indices are examined to yield outcomes, fungal disease occurrence, and mycotoxin contamination levels.

The insurance of the result's reliability lies in the continuous delivery of uniform experimental results, assuring that the results are reliable, precise, stable, and reproducible. The precision and reliability of the tools and devices used in the research, careful consideration in following data collection procedures, experimental setup, design, and treatments are premeditated from the inception to the culmination of the three-year field experiment to maintain consistency of the results.

Research indicates a complexity and variability in how applying individual nitrogen levels influences the severity of fungal ear rot and the consequent mycotoxin production. Furthermore, evidence points to affirmative relationships concerning hybrid resistance levels and contamination of mycotoxin. The application of nitrogen is critical for optimizing crop yield; utilizing hybrids with pronounced agronomic traits and tolerance levels can aid in accurately assessing the quantity of toxins produced to nitrogen application. Concurrently, the findings highlight that factors such as relative chlorophyll content and canopy reflectance indices, influenced by fertilization practices and hybrid genotypes, can play a pivotal role in strategizing advanced agricultural methods. It can facilitate better predictions and evaluations regarding the impacts on crop quality, the development of fungal diseases, and subsequent mycotoxin contamination in varying annual climatic conditions.

## **4.1 Yearly variation in agrometeorological conditions influences maize yield and mycotoxin contamination**

### **4.1.1 Agrometeorological conditions during the growing seasons**

Meteorological conditions affect agricultural productivity and plant growth and development by facilitating materials' transformation and the absorption of essential nutrients. These effects are mediated by climatic variables regulating water availability and the crop's thermal conditions. Key meteorological factors that influence precipitation, temperature, and relative humidity. The yearly agrometeorological conditions during the maize growing seasons of 2022, 2023, and 2024 are presented (figure 7.)

Maize, in particular, exhibits a marked sensitivity to ecological factors, with an adequate water supply identified as a critical determinant of its growth. This optimal water provision becomes especially vital during key growth phases (critical vegetative and generative phases) that contribute to the development and formation of yields in maize. The impact of water supply on maize yield is complex. It can be significantly modified by agro-ecological factors, such as soil characteristics and agro-technical practices, including hybrid selection and nutrient supply through fertilizer application and nutrient management, mainly using nitrogen fertilizers and other nutrient sources.

Maize growing seasons in Hungary span from April through September, covering a substantial period when the conditions are typically favorable. During these growth seasons, the soil exhibited suitable germination moisture content during sowing, fostering healthy crop growth and development. However, in the season of 2022, the months of May, June, and July were dry. The rainfall recorded was notably lower and below the 30-year average, instigating a crop growth and development deficit. Therefore, this necessitated supplementary irrigation to leverage the crop water requirement deficiencies to enhance crop performance. Two supplementary irrigations were performed in May and another in early July. These irrigation efforts were vital during the critical periods of the generative phase, such as flowering and grain filling, to ensure the maize plants received adequate water to support their growth and yield potential. Ensuring sufficient water during these key developmental stages maximizes crop productivity.

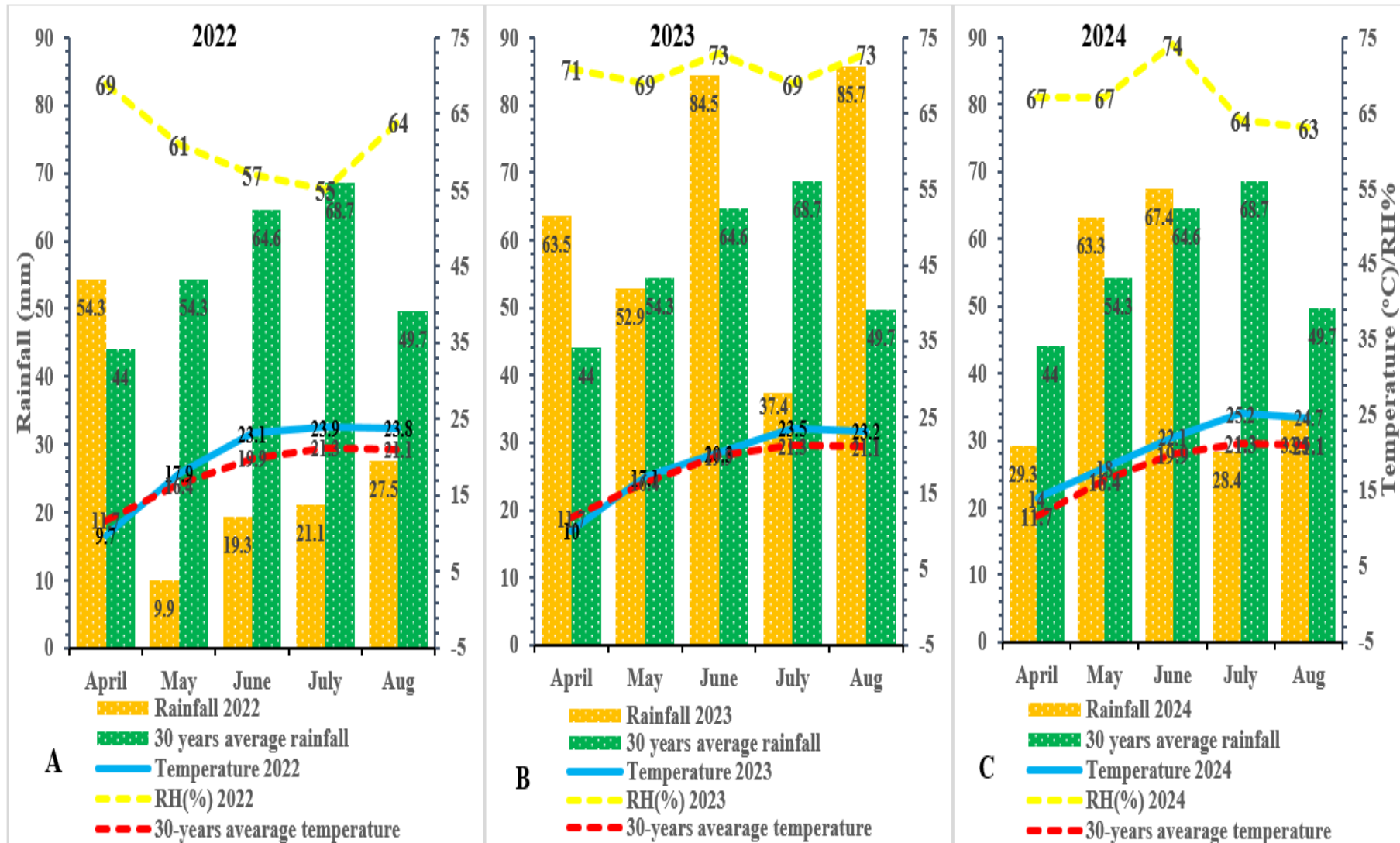


Figure 7: Yearly agrometeorological conditions during the growing seasons at the Latókép experiment site of the University of Debrecen (2022-2024): A, B, and C Rainfall, Temperature: and RH % 2022, 2023, and 2024 respectively).

The 2022 growing season was characterized by notably dry conditions, recording a total rainfall of only 132.1 mm. The subsequent seasons of 2023 and 2024 received significantly higher precipitation levels of 324 mm and 221.9 mm, respectively, marking 2023 as having achieved the highest rainfall among the three growing seasons (table 5). In the two latter seasons, the distribution of precipitation was more favorable for growth and development in 2024 than in 2023. The 2023 growing season received lower precipitation during early vegetative growth in May (V6) than in May 2024 (figure 4). Similarly, the precipitation in the later vegetative (VT) growth phase and early reproductive phases (R0-R1) in June was higher in 2023 than in 2024 at 84.7 and 67.4 mm, respectively, deviating by 17.1 mm. In July, during grain filling generative phases (R2-R4), both 2023 and 2024 received precipitation almost around deviating from each other by 9 mm (37.4 and 28.4 mm, respectively). However, during R5 and the maturation phase (R6) in August, experienced the highest precipitation in 2023, above the 30-year average as compared to 2024, deviating from each other by 52.2 mm, recording 85.7 mm in 2023 compared to 33.5 mm in 2024 (figure 7).

All the growing seasons, 2022, 2023, and 2024, experienced a temperature above the average of the past 30 years (Table 5), with a slight variation in the 2024>2022>2023 trend. Furthermore, the relative humidity (RH) during 2022 was at its lowest compared to subsequent years at 61.2 % compared to 71 and 67 % in the latter seasons, respectively.

*Table 5: Deviation of weather conditions in growing seasons (Debrecen, 2022-2024)*

Year	Total year rainfall	Rainfall in the growing season (mm)	Deviation from the 30 years. average	Temperature (°C)	Deviation from the 30 years. average	RH (%)
2022	297.20	132.10	-148.80	19.70	1.60	61.20
2023	615.40	324.00	42.70	18.80	0.70	71.00
2024	487.70	221.90	-59.40	20.80	2.70	67.00
30 years Average		281.30		18.10		

#### **4.1.2 Crop year and the interaction of hybrid selection and N rates influence grain yield and quality**

The fluctuation in weather patterns throughout the 2022-2024 growing seasons resulted in statistically significant differences ( $p<0.05$ ) regarding maize crop grain yield and quality. (Table 6). Specifically, yields in the dry season of 2022 were reduced by 40% and 63%

compared to those in 2023 and 2024 (Table 6). Further, grain protein content notably increased during the season characterized by low rainfall and reduced yields. In comparing 2022 to the subsequent years, there was a decline in grain protein levels of 32.7% for 2023 and 29.3% for 2024. Consequently, the suitability of crop years for promoting crop growth and yield was ranked as 2022 < 2023 < 2024, whereas the ranking for protein content was 2022 > 2024 > 2023.

*Table 6: Crop year weather variability influences grain yield and quality*

Source of variation	Yield (kg ha <sup>-1</sup> )	Starch (%)	Protein (%)
2022	7638	71.50	10.60
2023	12793	64.34	7.13
2024	20755	64.81	7.50
<i>LSD</i> ( <i>0.05</i> )	779.00	0.98	0.35

The favorable rainfall conditions, combined with nearly optimal agrotechnology practices and the higher-than-average temperatures experienced during the 2023-2024 growing years, resulted in a remarkably favorable yield in the years to the year 2022 (table 6). The differences in yield align with other studies indicating that water deficits in the growing season reduce yield, and growing season with adequate water attains higher yield potentials attributed to water supply (Bramdeo and Rátonyi, 2020; Pepó, 2012). Although the 2023 growing season experienced the highest rainfall than 2024, the latter gained the highest yield. It could be attributable to an indirect effect of soil water balance brought in by dryness, causing soil water deficits in the previous growing season, 2022. Wang et al., 2022; Széles et al., 2018 reports the effect of soil water deficit extremes in a preceding year could be influential to the subsequent season. Thus, 2024 growing had leveraged soil water for crop uptake and facilitated nutrient availability compared to 2023. Further, the air temperature greater than the 30-year average, favoring 2024 in 2024>2022>2023 trend, could have been attributed to the yield differences between 2023 and 2024. The increased yield in 2024 is partly favored by the temperature being relatively close to the optimum for assimilation and photosynthesis during grain filling (Sánchez et al., 2014).

Simultaneously, the interacting influence on yield by the year and agrotechnical practices was evaluated (Figure 8). Bojtor et al. (2021) stressed that yield is affected by interactions between crop year variations, nitrogen fertilization practices, and hybrid selection strategies.

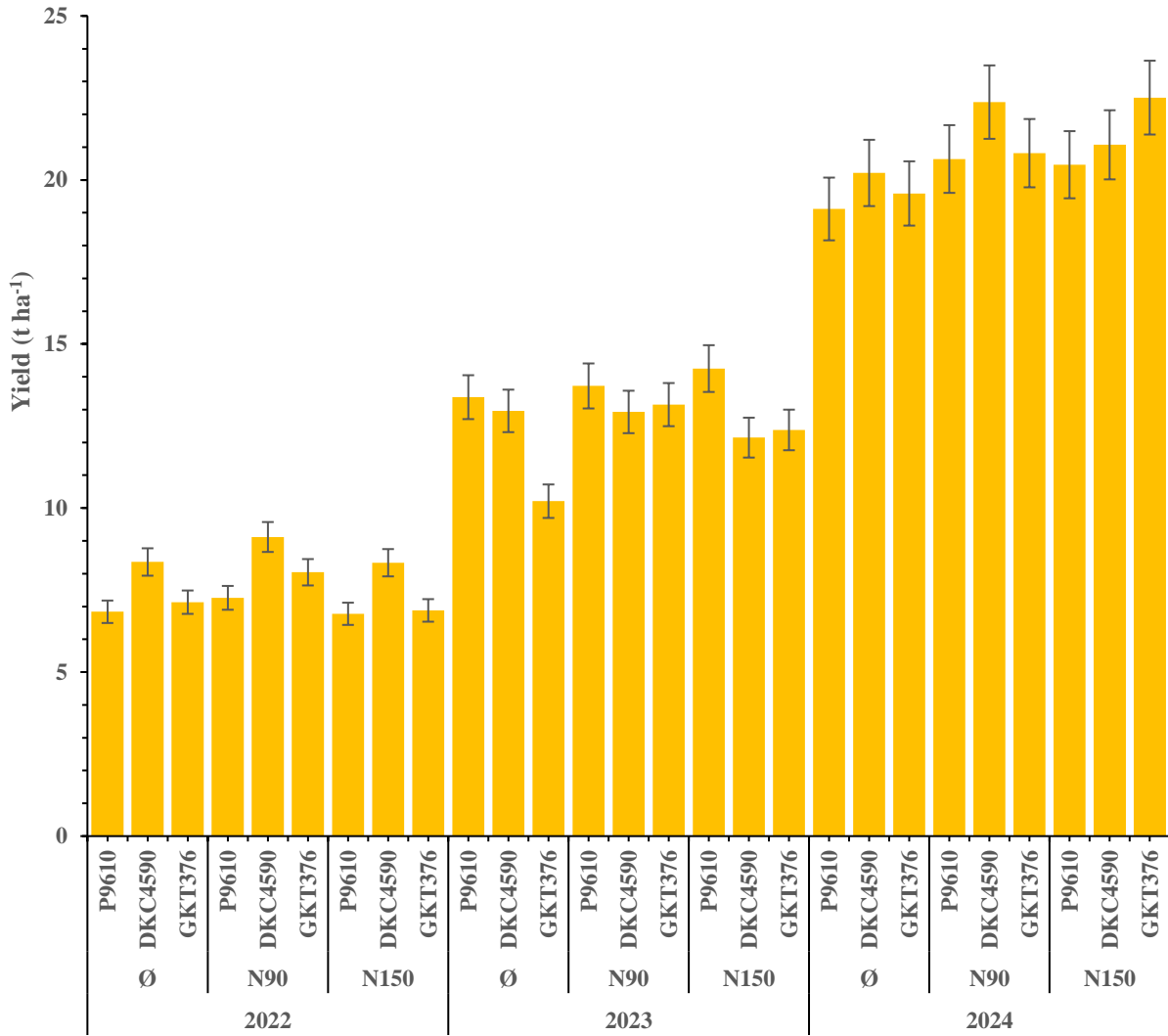


Figure 8: Interactive influence of N dosage and selected hybrids on yield across the experiment years (Debrecen, 2022-2024)

Considering that fertilization management can bridge the gap of the positive effect of crop year variation on maize yield (Qiao et al., 2021; Liu et al., 2021), the current study was contrary, demonstrating crop year to be a decisive factor. This is evidenced by the superior performance of all hybrids in 2024 compared to 2023 and 2022 under nitrogen treatment in 2024>2023>2022 (Figure 8). However, the maize hybrid affected nitrogen efficiency, enabling the system to sustain higher yield potential. This is demonstrated by the significant ( $p<0.05$ ) superior performance of hybrid DKC4590 in the unfavorable year 2022 in all N levels. However, the variability in the succeeding years was differential among the selected hybrids. In 2023, among the various N-level treatments, the lowest significant yield ( $p<0.05$ ) was recorded in the unfertilized control (N0), with 10.2  $\text{tha}^{-1}$  for variety GKT 376 compared to no variation between DKC4590 and P9610 at 13.4 and 13  $\text{tha}^{-1}$  respectively.

Additionally, the 2022 best-performing hybrid DKC4590 was significantly surpassed by hybrid P9610, particularly at the recommended rate of N150 (Bojtor et al., 2021). In 2024, the results showed no substantial variations between hybrids within varying individual N rates; however, variation existed between hybrids compared to other N treatments. Therefore, the interaction between well-suited agronomical practices such as nitrogen fertilization, maturity group, and maize genotypes, which respond to unpredictability in growing season conditions, may provide optimal yields compared to non-interacting traits. In optimizing one of these components, understanding all possible interactions and synergistic effects is needed to avoid unintended trade-offs.

This study showed measurable effects of nitrogen fertilization and hybrid choice and their interactions on maize grain yield in response to crop year variations (Figure 7). The hybrid response to nitrogen over the years indicates that GKT376 exhibits a degree of instability and is significantly affected by nitrogen fertilization across different crop years. In contrast, the hybrids DKC4590 and P9610 demonstrate a more stable performance during the 2023 growing season. In the 2023 growing season, N fertilization sharply affected the yield of GKT376 to other hybrids on the non-fertilized control and other N treatments. Further, GKT376 indicated the highest performance at N90 in 2022 and 2023; the differences between the controls in 2022 and 2023 are 1 ton and 3 tons, respectively. However, in the 2024 growing season, the highest performance for GKT376 was recorded at the highest fertilizer rate used (N150).

Excluding the growing season of 2022, in which hybrid DKC4590 exhibited a marked superiority over other hybrids (P9610 and GKT376) across all nitrogen (N) treatments, hybrids DKC4590 and P9610 demonstrated relative stability with varying N applications in the subsequent years of 2023 and 2024. A significant difference ( $P < 0.05$ ) between DKC4590 and P9610 emerged only at the N150 dosage in 2023, wherein P9610 outperformed DKC4590. Nevertheless, this yield advantage for P9610 was not statistically significant when evaluated against the yields from all hybrids at the N90 level, suggesting the best performance of all hybrids at N90. Consequently, this analysis underscores the necessity for adaptive nitrogen fertilization and the selection of suitable hybrids to optimize maize production strategies, particularly in light of unpredictable climatic fluctuations and

yearly yield variations. It is hard to plan an adaptive N fertilization when you hardly know how the coming year will be; it can be simple to do this ex-post, but probably not ex-ante. However, knowing the amount of soil water reservoir before sowing determines the amount of N that can be applied in split achieved by soil sampling prior to cultivation. Additionally, a participatory integrated climate service approach using the nationwide year weather forecast and area-downscaled monthly and quarterly forecasts can supplement planning for adaptive nitrogen fertilization, particularly in rainfed crop production.

This argument is bolstered by the superior yield performance of hybrid DKC4590 at the N90 level during 2022 and 2024, alongside the absence of significant yield differences between the N90 and N150 treatments in 2023. Therefore, comprehensive assessments are essential considering all relevant factors influencing this adaptability.

This study substantiates these determinants by considering nitrogen fertilization practices that depend on hybrid selection. In this scenario, further field experiments on hybrid choice that are authenticated in a more comprehensive and integrated context to assess from an integrated approach the basis of yield potential with conventional technologies seem necessary to avoid an extremely narrow focus on eco-physiological patterns. Further, crop year showed a tremendous influence on the yield of maize, affecting nitrogen fertilization regimes and the selected hybrids. Thus, it is a cornerstone to understand that each crop year brings unique characteristics and factors that can significantly affect the final yield. Hence, carefully considering each crop year's specific conditions and requirements could enable farmers to make informed decisions regarding the optimal timing and dosage of nitrogen fertilization and the most suitable hybrids to cultivate. Through a comprehensive understanding and analysis of the crop year, farmers can maximize their maize yield while ensuring efficient resource allocation and sustainable agricultural practices.

#### **4.1.3 Yearly variation in agrometeorological conditions influences fungal disease development and mycotoxin contamination**

The effect of annual agrometeorological conditions on the development of fungal diseases and mycotoxin contamination is illustrated in Table 7. Additionally, Table 8 provides a detailed analysis of the complex interactions, as evidenced by Pearson's correlations,

between yearly variations and weather elements concerning ear rot infection and mycotoxin contamination.

*Table 7: Effects of year variability on fungal disease development and mycotoxin contamination*

Source of variation	Ear rot severity			Mycotoxins contamination		
	AF%	FV%	FG%	AFB1(ppb)	FBs(ppm)	DON(ppm)
2022	0.17	0.16	11.50	40.28	2.15	4.06
2023	0.18	0.19	24.50	<LOD	2.71	56.59
2024	0.32	0.47	0.74	36.35	2.73	0.63
<b>LSD(0.05)</b>	0.06	0.08	2.85	20.73	0.75	14.23

AF%= percentage of kernels damaged by *A. flavus*; FV%= percentage of kernels damaged by *F. verticillioides*; FG%= percentage of kernels damaged by *F. graminearum*; AFB1= aflatoxin B1; FB= fumonisins B1+B2; DON= deoxynivalenol

Significant ( $p < 0.05$ ) strong positive and negative relationships existed between various variables. Firstly, the crop year strongly and positively influenced yield, confirmed by the obtained yield showing significant ( $p < 0.05$ ) yield increase between years with varying weather conditions, specifically, precipitation suitability (Tables 5 and 6). The individual agrometeorological conditions during the growing seasons positively or negatively affected the development of fungal disease and mycotoxin contamination.

Rainfall indicates a positive influence on FG% ( $r = 0.578$ ), DON production ( $r = 0.858$ ), and FBs production ( $r = 0.830$ ). Years with higher rainfall indicated higher FG % and heightened DON production (Tables 5 and 7). However, precipitation indicated no association with AF % and FV% ( $r = 0.021$  and  $0.037$ ), respectively, but negatively affected the production of AFB1 ( $r = -0.922$ ). This study shows that AFB1 production was recorded as negligible in 2023, the year with the highest rainfall, and the heightened amount of AFB1 was recorded in 2022, the year with the lowest rainfall, followed by 2024. Temperature influence is pronounced in its negative effects on FG % ( $r = -0.922$ ) and DON production ( $r = -0.855$ ). Further, temperature demonstrated a positive relationship with AF% ( $r = 0.874$ ), FV% ( $r = 0.866$ ) and AFB1 production ( $r = 0.773$ ). Subsequently, relative humidity (RH %)-demonstrated positive, negative, and no relationships with fungal disease development and mycotoxin contamination. Regarding fungal disease development, RH% showed no correlation with AF% and FV% and a positive correlation with FG% ( $r = 0.455$ ). However, it strongly negatively ( $r = -0.857$ ) correlated with AFB1 production, indicating strong relationships with both fusarium mycotoxins FBs ( $r = 0.901$ ) and DON ( $r = 0.775$ ).

Table 8: Correlation coefficient values showing a relationship between yearly agrometeorological conditions on yield, fungal disease development, and mycotoxin contamination (Debrecen, 2022-2024)

	Yield kg ha <sup>-1</sup>	Rainfall (mm)	Temp (°C)	RH (%)	AF%	AFB1	FBs	DON		
Yield kg ha <sup>-1</sup>	1.00									
Rainfall (mm)	0.36	1.00								
Temp (°C)	0.65	-0.48	1.00							
RH (%)	0.48	0.99	-0.35	1.00						
AF%	0.94	0.02	0.87	0.16	1.00					
FV%	0.95	0.04	0.86	0.18	1.00	1.00				
FG%	-0.56	0.58	-0.99	0.45	-0.80	-0.79	1.00			
AFB1(ppb)	0.03	-0.92	0.78	-0.86	0.37	0.35	-0.85	1.00		
FBs(ppm)	0.82	0.83	0.09	0.90	0.58	0.59	0.02	-0.55	1.00	
DON(ppm)	-0.18	0.86	-0.86	0.78	-0.50	-0.48	0.92	-0.99	0.42	1.00

RH% = Relative humidity (%); AF%= percentage of kernels damaged by *A. flavus*; FV%= percentage of kernels damaged by *F. verticillioides*; FG%= percentage of kernels damaged by *F. graminearum*; AFB1= aflatoxin B1; FB= fumonisins B1+B2; DON= deoxynivalenol

Occurrence and interactions of the meteorological conditions bring a range of unique characteristics in the year that either accelerate or provide unfavorable conditions for colonization, disease development, and synthesis of mycotoxins. The range includes dry and warm conditions, moderate rainfall and warm conditions, and higher precipitations with mild temperatures (Mesterhárzy et al., 2022). In the current study, fungal disease development and mycotoxin contamination were highly influenced by the variability in the weather conditions in the growing season ( $p < 0.05$ ). AF% and FV% by *A. flavus* and *F. verticillioides* exhibited no differences in 2022 and 2023 while recording significantly elevated infections in 2024. Mycotoxin fumonisins (FBs) production by *F. verticillioides* was non-significant in all three growing seasons and below European maximum tolerable levels (MTL). On the contrary, AFB1 production by *A. flavus* was not recorded in the wettest year, 2023, with the highest relative humidity (RH %). The highest amount of AFB1 was recorded in the 2022 season, characterized by dry conditions, confirming the previous study showing that reduced rainfall and warmer conditions facilitate colonization of *A. flavus* and respective mycotoxin contamination, corroborating Giorni et al. (2016). Further, Kos et al. (2024) reported that 94% of maize samples collected in 2012 were contaminated with aflatoxin; the year noted extreme drought in Serbia, exhibiting concentration levels ranging from 0.6 to 205  $\mu\text{g kg}^{-1}$ .

On the other hand, FG% by *F. graminearum* was significant and highest in 2023, the wet year with higher RH%. Consequently, the weather conditions in 2023 favored the production of deoxynivalenol (DON) besides fungal colonization and disease development, corroborated by Bhatnagar et al. (2014). Given the heightened FV% in 2024 compared to other years, there were no significant differences in the associated FBs (table 6). This indicates that the variability in weather conditions during this study did not influence the production of fumonisin toxins.

## 4.2 Crop year, hybrids, and nitrogen fertilization harness relative chlorophyll contents and canopy reflectance indices concerning yield, fungal disease development, and mycotoxin contamination

### 4.2.1 Yield response to relative chlorophyll content dynamics in respective hybrids, n rates, and crop year.

The results significantly differed ( $p < 0.05$ ) in relative chlorophyll content between maize hybrids and N rates associated with the final grain yield (Table 9). Relative chlorophyll content differences existed among maize hybrids at different growth stages, regardless of specific nitrogen rate and crop year.

Table 9: Relative chlorophyll content (SPAD) in different growth stages to nitrogen rates and maize hybrids on grain yield in the experiment years (Debrecen: 2022-2024)

Source of variation	Growth stage and (SPAD)					Yield (Kg/ha)	Protein (%)	Starch (%)	
	V6	VT	R0-R3	R6	AVG				
<b>Nitrogen fertilization rates</b>									
2022	Ø	44.55	54.82	54.97	47.88	50.55	7442	9.05	73.19
	N90	47.26	57.29	57.02	52.63	53.55	8141	11.17	69.91
	N150	48.44	58.16	59.26	53.9	54.94	7330	11.58	71.40
2023	Ø	45.56	57.17	54.56	45.71	50.75	12183	6.73	64.66
	N90	44.77	60.1	57.22	45.99	52.02	13268	7.20	64.52
	N150	48.68	61.28	58.92	46.86	53.94	12926	7.45	63.84
2024	Ø	41.41	60.01	60.68	45.71	51.95	19640	7.376	64.8
	N90	39.58	60.24	62.05	50.15	53.01	21276	7.716	64.49
	N150	40.55	61.38	61.97	50.41	53.58	21349	7.397	65.14
	<b>LSD<sub>(0.05)</sub></b>	<b>2.63</b>	<b>1.784</b>	<b>1.868</b>	<b>3.555</b>	<b>1.256</b>	<b>1374.6</b>	<b>0.5828</b>	<b>1.822</b>
<b>Maize Hybrid effects</b>									
2022	P9610	47.36	58.15	57.53	49.49	53.13	6960	10.63	72.29
	DKC4590	46.69	56.47	56.92	52.44	53.13	8602	9.79	71.72
	GKT376	46.2	55.66	56.8	52.48	52.78	7351	11.38	70.48
2023	P9610	48.7	59.74	55.92	44.91	52.32	13784	7.27	64.32
	DKC4590	44.85	57.86	55.27	45.66	50.91	12679	6.82	64.94
	GKT376	45.45	60.95	59.52	48.01	53.48	11914	7.30	63.76
2024	P9610	41.88	62.31	62.25	46.73	53.29	20073	7.407	65.51
	DKC4590	39.07	57.95	60.29	48.84	51.54	21220	7.27	64.83
	GKT376	40.58	61.37	62.17	50.71	53.71	20972	7.811	64.09
	<b>LSD<sub>(0.05)</sub></b>	<b>2.572</b>	<b>1.802</b>	<b>1.78</b>	<b>2.873</b>	<b>0.987</b>	<b>1231.9</b>	<b>0.6144</b>	<b>1.795</b>

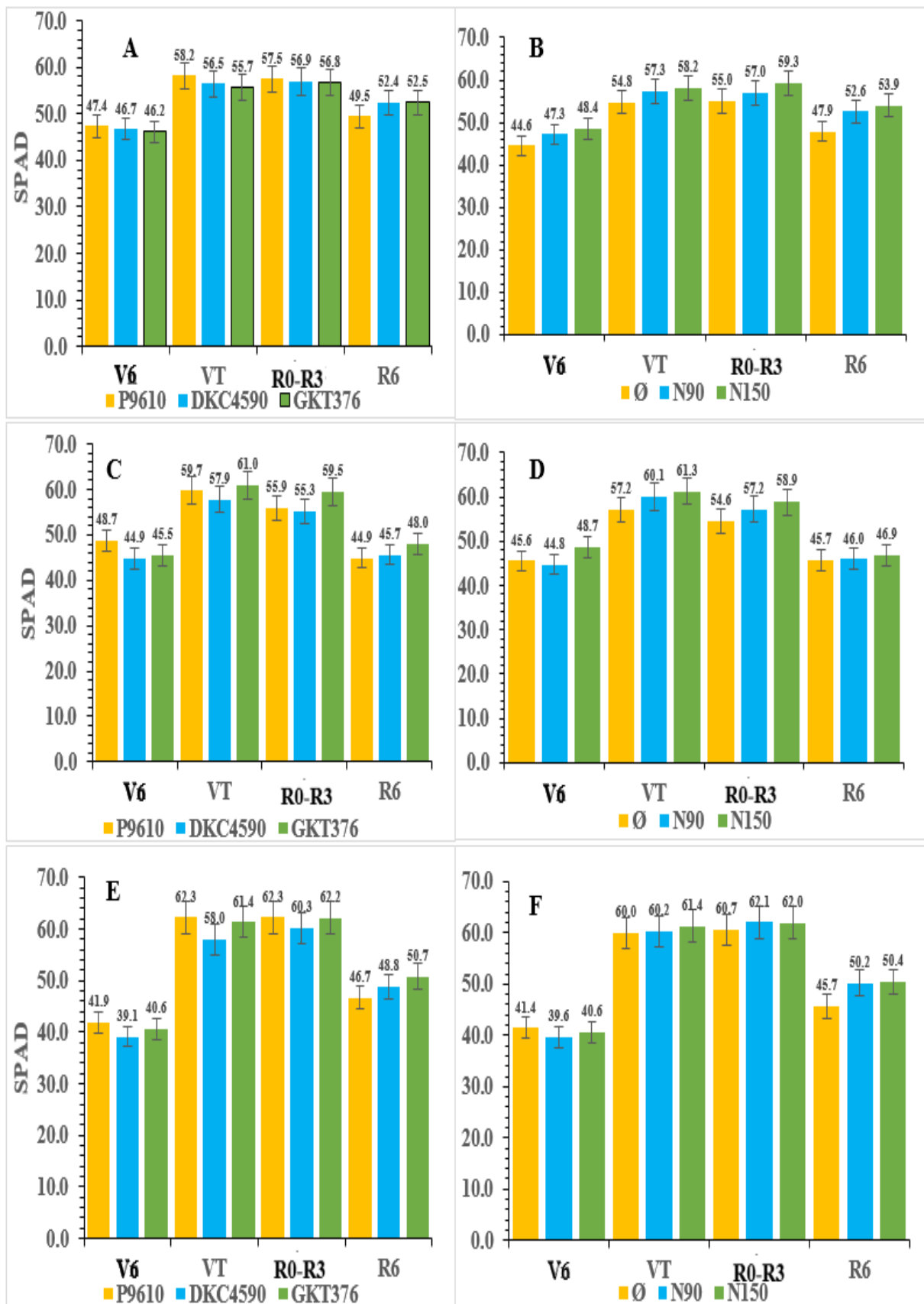


Figure 9: Effects of nitrogen fertilization and hybrid selection on relative chlorophyll content-SPAD in various growth stages (A&B; C&D; E&F) in 2022, 2023, and 2024, respectively (Debrecen, 2022-2024)

The SPAD index between N rates and hybrids positively influenced yield between years. Similarly, the crop displayed the lowest and highest SPAD index between growth stages during V6, R6, and VT, R0-R3, respectively (Figure 9). R0-R3 indicated no variations; hence, they were grouped. At V6, the SPAD index between years recorded in 2024 was significantly ( $p < 0.05$ ) lower than the records in 2022 and 2023 in response to nitrogen and hybrids. Additionally, within years, the SPAD index recorded in 2024 was not significant ( $p > 0.05$ ) between nitrogen treatment and selected hybrids but was significant ( $p < 0.05$ ) in 2022 and 2023. Conversely, during the VT and R0-R3 growth stages, 2024 recorded the highest significant values between 2022 and 2023. However, within years, VT and R0-R3 values of the SPAD index were significantly affected by N rates in 2022 and 2023 while non-significant in 2024. The influence increased with N rates, although the same indicated no effects regarding hybrids selected. Additionally, the impact of N rates in the growth stages indicates no variation in SPAD values between N90 and N150, similar to hybrids.

The results showed that, in all cropping years, particularly during VT and R0-R3 stages, the SPAD index was significantly ( $p < 0.05$ ) higher and affected by year in response to nitrogen treatment and hybrid selection. The influence in yield is expressed by the fact that the leaf chlorophyll reacts to environmental changes as a light energy receptor in photon capturing and an electron donor in the photosynthesis process, and it is essential in the technical evaluation of the photosynthesis capacity (Ahmad et al., 2019). Therefore, under optimal leaf chlorophyll content, the photosynthesis process maximizes, improving the dry matter production of the vegetative structures and intensifying the quantitative points and final yield (Yan et al., 2021). Under N rates, the chlorophyll content levels indicate the most adequate plant development stage for the best utilization of nitrogen, which is directly related to final production. This is verified by the current study, which aligns with Shapiro et al. (2016), suggesting that stages with higher SPAD indexes facilitate maximized N nitrogen utilization. The current study reveals that the nitrogen treatments N90 and N150 did not exhibit substantial differences, particularly when compared to the lower SPAD values associated with the control treatment N0. However, on the contrary, the hybrid that showed better average SPAD across the years (P9610) during VT did not demonstrate better average yield, being surpassed by hybrid DKC4590 with no significant variation with

GKT376. It indicates nitrogen as a decisive factor, making the general rank of factors that affected yield as Year>N rates>Hybrids.

The SPAD index pigment content can serve as a metric for calibrating nitrogen fertilization rates, using the technology of chlorophyll meter reading, and allows for more rapid and accurate application as an indicative resource of the nitrogen nutritional status in crops (Melash et al., 2023). Some studies have shown a strong relationship between N levels and chlorophyll content in predicting the apparent photosynthesis rate in the initial stage of maize plant and final yield production (Yan et al., 2021; Wang et al., 2021). However, this study suggests that the VT growth stage influences and maximizes N use. Therefore, the SPAD index transforms into an accurate, supportive technique, optimizing the results to predict the apparent photosynthesis rate and the final production.

#### **4.2.2 Canopy reflectance indices influenced by N rates and Selected Hybrids across various growth stages**

The leaf area index (LAI) indices across various growth stages were significant ( $p<0.05$ ) within hybrids and N rates in the years. Similarly, Normalized Difference Vegetation Index (NDVI) values were significantly different ( $p<0.05$ ) between the measurements in various growth stages in response to nitrogen fertilization and hybrids in the years (Table 10)

The various growth stages of the crops exhibited pronounced impacts on LAI and NDVI values, characterized by diminishing return associations as the crop progressed through its growth phases. These indices reached their minimum levels during the V6 and R6 stages while peaking at the VT and R0-R3 stages, particularly highlighted by the effects of N rates and selected hybrids in the experimental years (Figures 10, 11). Notably, in 2022, LAI demonstrated the highest significant ( $p<0.05$ ) values during the R0-R3 stages at a nitrogen rate of N50 and for the DKC 4590 hybrid. In 2023, the peak significant LAI values were observed during the VT stage, influenced by varying nitrogen rates.

Table 10: Effects of N fertilization and selected hybrids on NDVI and LAI in various growth stages to yield and grain quality

Source of variation	Growth stage and LAI					Growth stage and NDVI					Yield (Kg/ha)	Protein (%)	Starch (%)	
	V6	VT	R0-R3	R6	AVG	V6	VT	R0-R3	R6	AVG				
<b>Nitrogen fertilization rates</b>														
2022	Ø	1.15	3.24	3.71	1.94	2.51	0.47	0.68	0.73	0.47	0.59	7442	9.05	73.19
	N90	1.60	3.81	3.88	2.33	2.90	0.56	0.72	0.75	0.53	0.64	8141	11.17	69.91
	N150	1.66	3.92	4.27	2.34	3.05	0.55	0.71	0.73	0.51	0.62	7330	11.58	71.40
2023	Ø	2.20	3.18	2.59	2.22	2.55	0.47	0.77	0.73	0.46	0.61	12183	6.73	64.66
	N90	2.35	3.59	3.21	2.52	2.92	0.45	0.80	0.74	0.51	0.63	13268	7.20	64.52
	N150	2.44	3.87	3.33	2.70	3.08	0.56	0.81	0.73	0.47	0.65	12926	7.45	63.84
2024	Ø	1.43	3.42	4.77	2.22	2.96	0.69	0.79	0.71	0.46	0.66	19640	7.376	64.8
	N90	1.55	3.57	5.34	2.38	3.21	0.70	0.78	0.76	0.47	0.68	21276	7.716	64.49
	N150	1.30	3.36	4.31	2.67	2.91	0.67	0.79	0.77	0.48	0.68	21349	7.397	65.14
	<b>LSD<sub>(0.05)</sub></b>	<b>0.2800</b>	<b>0.2622</b>	<b>0.3793</b>	<b>0.3892</b>	<b>0.1720</b>	<b>0.0565</b>	<b>0.0296</b>	<b>0.0201</b>	<b>0.0574</b>	<b>0.0256</b>	<b>1374.6</b>	<b>0.5828</b>	<b>1.822</b>
<b>Maize Hybrid effects</b>														
2022	P9610	1.31	3.49	3.84	2.10	2.68	0.49	0.68	0.72	0.47	0.59	6960	10.63	72.29
	DKC4590	1.66	3.82	4.10	2.38	2.99	0.55	0.76	0.74	0.54	0.65	8602	9.79	71.72
	GKT376	1.44	3.67	3.92	2.13	2.79	0.54	0.68	0.74	0.50	0.61	7351	11.38	70.48
2023	P9610	2.21	3.50	2.95	2.47	2.78	0.43	0.80	0.71	0.45	0.60	13784	7.27	64.32
	DKC4590	2.39	3.58	3.14	2.48	2.90	0.56	0.80	0.75	0.54	0.66	12679	6.82	64.94
	GKT376	2.40	3.57	3.05	2.49	2.88	0.50	0.79	0.74	0.45	0.62	11914	7.30	63.76
2024	P9610	1.31	3.40	4.83	2.39	3.02	0.72	0.79	0.74	0.46	0.68	20073	7.407	65.51
	DKC4590	1.48	3.44	4.72	2.49	2.99	0.67	0.78	0.75	0.51	0.68	21220	7.27	64.83
	GKT376	1.49	3.51	4.87	2.40	3.07	0.67	0.79	0.76	0.44	0.67	20972	7.811	64.09
	<b>LSD<sub>(0.05)</sub></b>	<b>0.3887</b>	<b>0.2804</b>	<b>0.3462</b>	<b>0.2695</b>	<b>0.2080</b>	<b>0.1106</b>	<b>0.0334</b>	<b>0.0254</b>	<b>0.0354</b>	<b>0.0379</b>	<b>1231.9</b>	<b>0.6144</b>	<b>1.795</b>

However, all hybrids reached their maximum LAI at this stage without achieving statistical significance ( $p>0.05$ ). In contrast, the 2024 data illustrated that nitrogen fertilization had a significant impact, with the highest LAI recorded during the R0-R3 stages at a nitrogen rate of N90. Similarly, the highest LAI values remained statistically non-significant for all selected hybrids during the R0-R3 stages. However, LAI significant ( $p<0.05$ ) variation was observed between various growth stages.

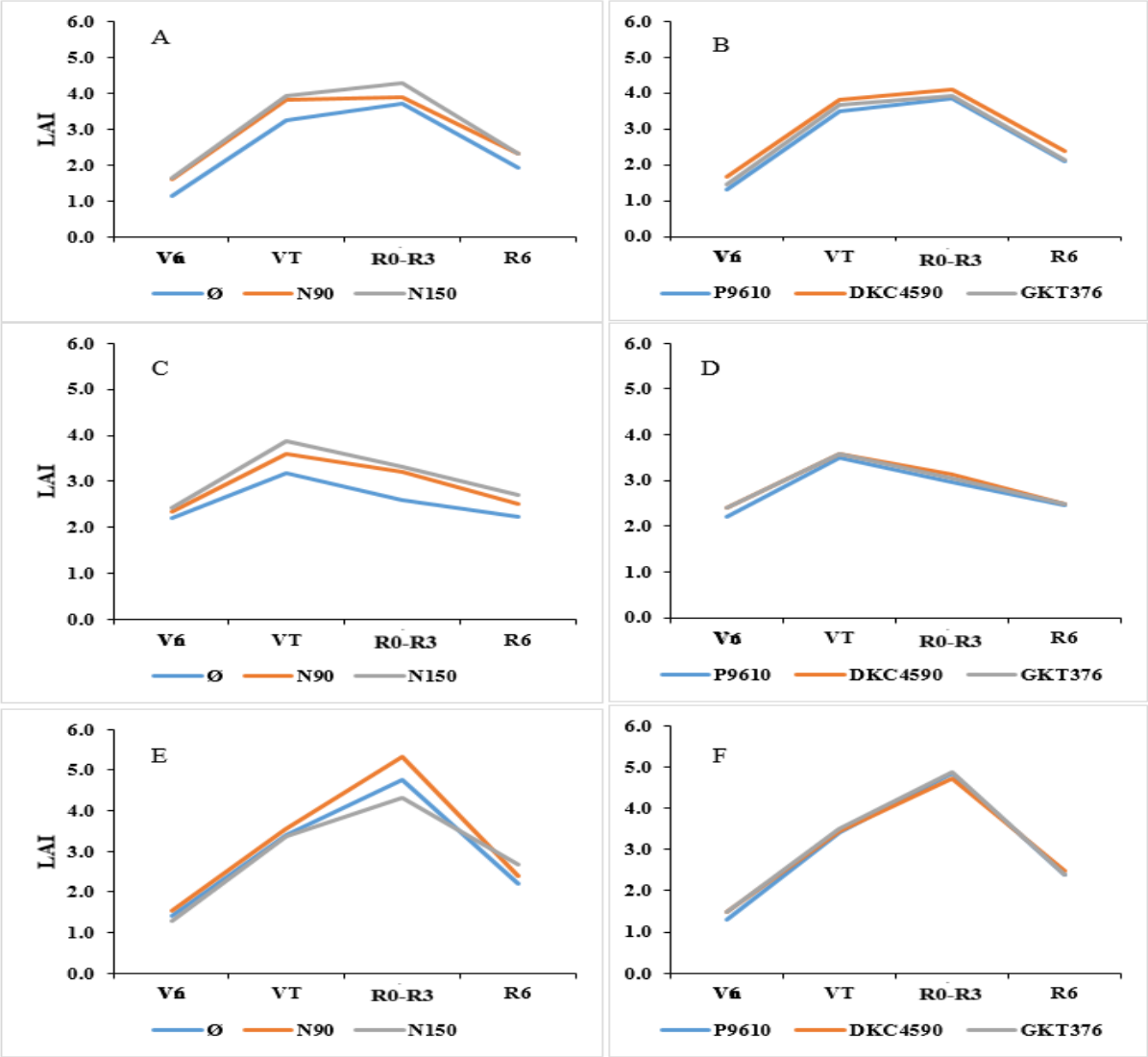


Figure 10: Nitrogen fertilization and Hybrids influence LAI in various growth stages (A&B; C&D; E&F) in 2022, 2023, and 2024, respectively

Similarly, NDVI indices showed the highest values during the VT and R0-R3 growth stages and the lowest values during the V6 and R6 growth stages in response to nitrogen rates and maize hybrids over the years. In all years, The NDVI values recorded at VT and R0-R3

stages were non-significant ( $P>0.05$ ) except at VT for hybrids in 2022 (Figure 11B and Table 9); however, there existed significant variation during V6 and R6 stages in response to N rates and maize hybrids.

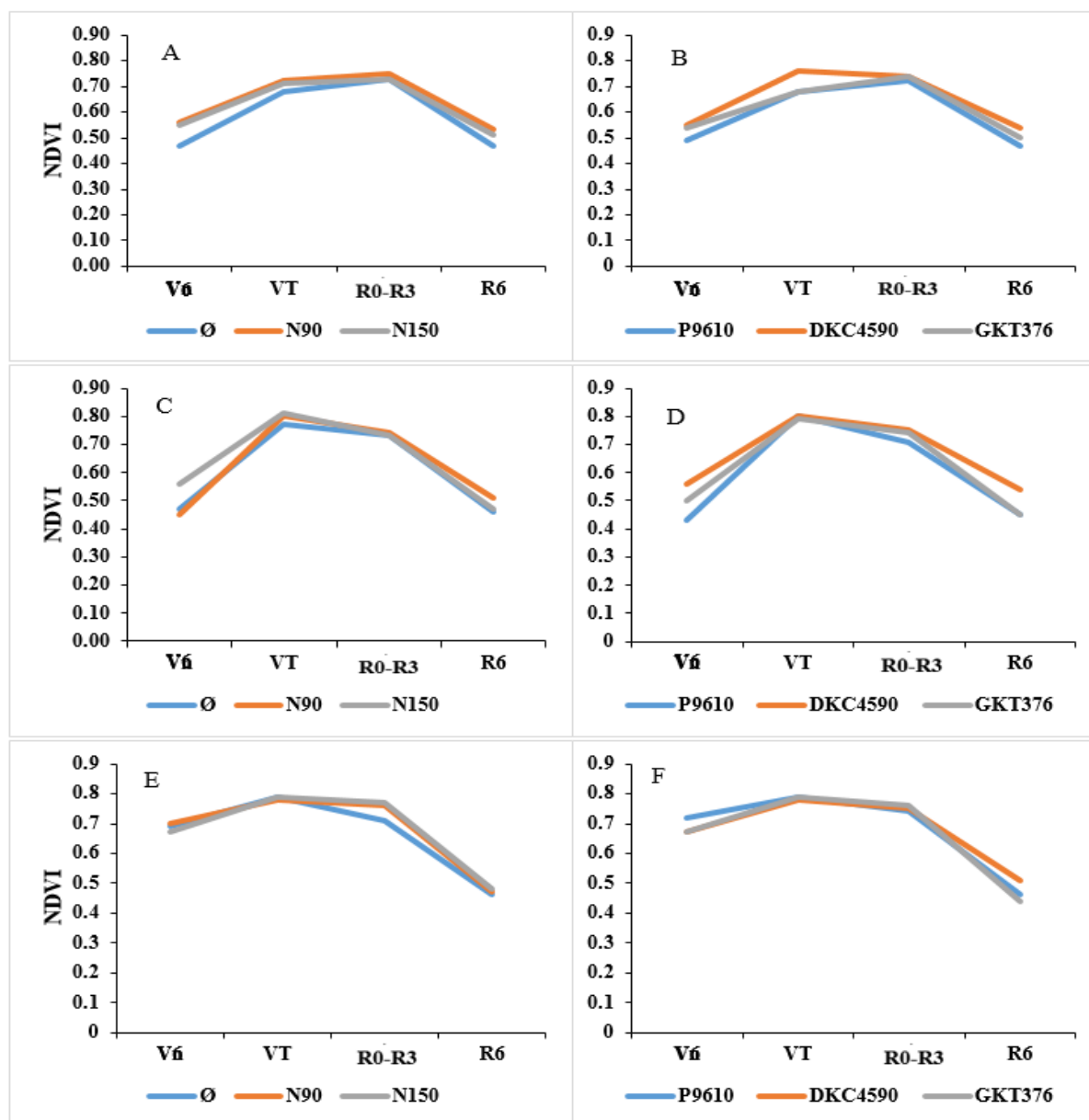


Figure 11: Nitrogen fertilization and Hybrids influence NDVI in various growth stages (A&B; C&D; E&F) in 2022, 2023, and 2024, respectively

The interactive effects between N rates, hybrids, and crop years on LAI and NDVI were examined (Figures 12 and 13). In 2022, LAI ranged from 0.99 at N0 and V6 (P9610) to 4.36 at N150 and R0-R3 (GKT376), while in 2023, values showed a range of 2.14 at N0 and V6 (P9610) to 3.92 at N150 and VT (DKC4590). In 2024, LAI values recorded non-significant lowest values during V6 across all interacting examined factors; the highest and

significant ( $P < 0.05$ ) values were recorded during R0-R3 growth stages noting GKT 376 (N90) with the highest value of 5.51 and the lowest value of 4.1 for DKC4590 at N150. Generally, crop year affected the LAI trend, indicating the V6 stage record of the lowest values than all stages; however, the values were significantly higher in 2023 than in 2022 and 2024, irrespective of N rate and hybrids.

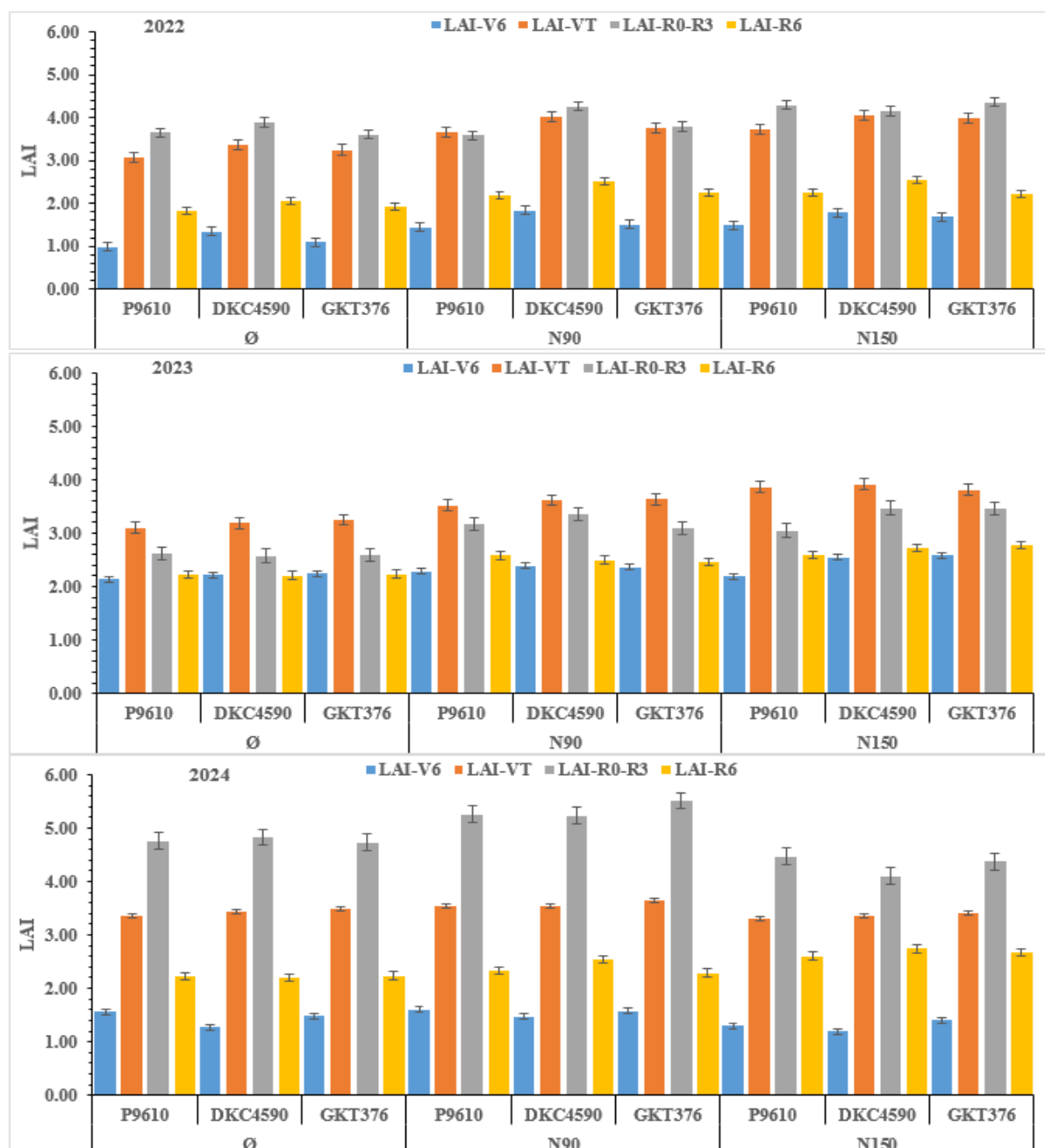


Figure 12: Interactive effects of crop year, nitrogen fertilization, and hybrids on leaf area Index (LAI): Debrecen, 2022-2024)

Unlike LAI, the NDVI at V6 generally indicated significant differences ( $P < 0.05$ ) lower in 2023 than in 2022 and 2024, except at N150 (DKC4590). The lowest values were recorded

during the R6 stage at 0.37 at N150 (P9610), while the higher and significant values between stages were recorded during VT. In each year, 2022 recorded the lowest NDVI value of 0.41 during V6 at N0 (P9610) and the highest value of 0.76 during VT at N150 (DKC4590). In 2023, the lowest NDVI record was 0.37 at N150 (P9610) during R6, and the highest record was 0.82 during VT at 150 (P9610), making P9610 at N150 showing the highest and lowest values during R6 and VT, respectively. In 2024, there were no significant differences between the NDVI values across N rates and hybrids within growth stages V6, VT, and R0-R3, but significant between the growth stages. However, there were significant differences (P9610) within growth stage R6, with the lowest value of 0.41 at N0 (GKT376).

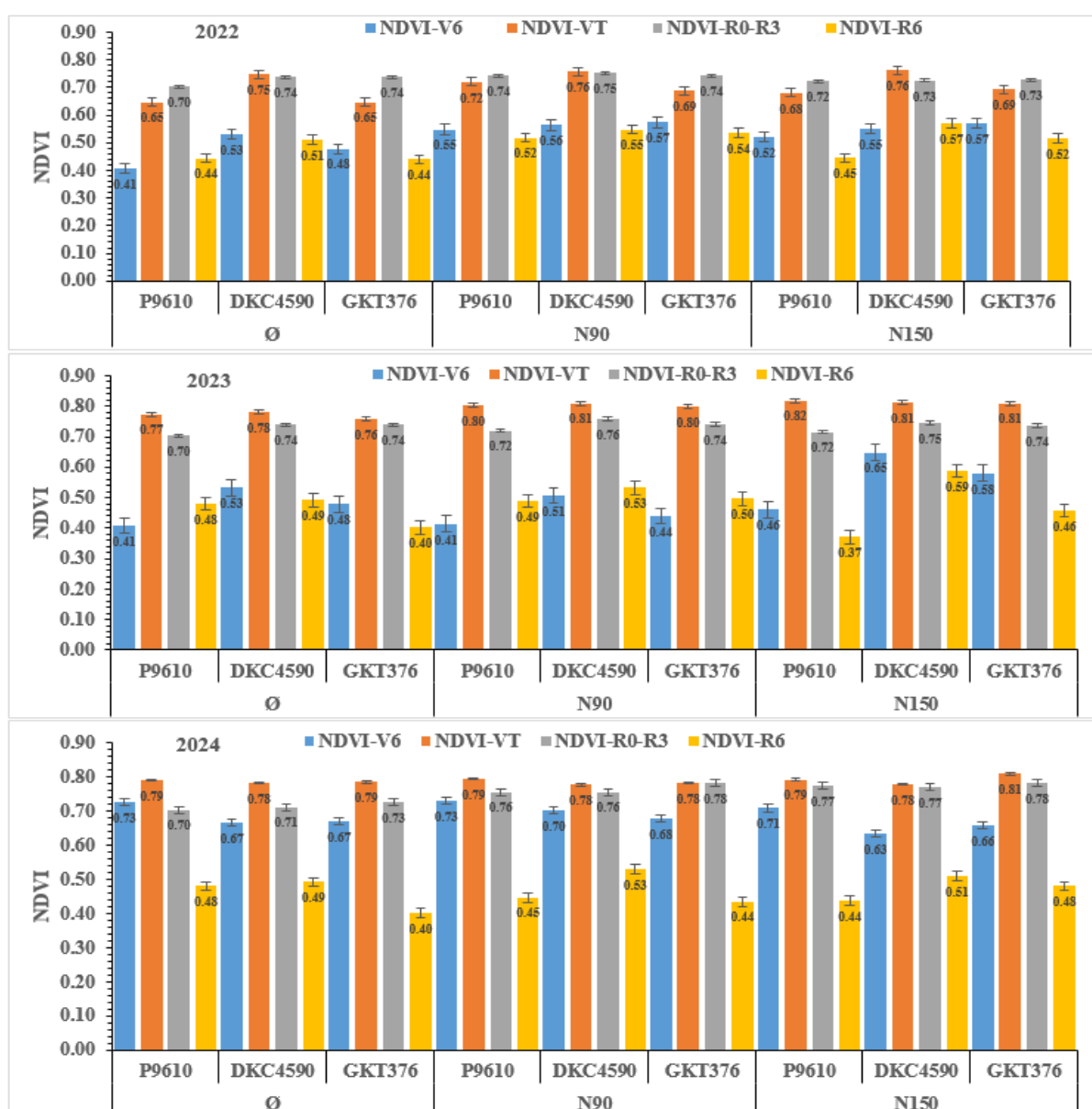


Figure 13: Interactive effects of crop year, nitrogen fertilization, and hybrids on NDVI (Debrecen, 2022-2024)

The assimilative capacity of a crop depends upon the balance between the quantity of incoming light and the amount that exits the crop, which influences photosynthetic capacity and production (Dahiya et al., 2023). In most crops, leaf photosynthesis contributes to 95% of the total dry matter harvested; the only exception is the mineral nutrients, representing the remaining 5% of the overall plant mass (Chen, 1994; Dong et al., 1997 in Li et al., 2022). A critical factor affecting this assimilative efficiency is the leaf area index (LAI), which significantly affects the crop's capacity to assimilate and overall productivity (Bakó et al., 2025). LAI is influenced by genotype, soil conditions, and climate. Consequently, NDVI serves as an indirect method of reflectance measurement that has been utilized to estimate both plant biomass and yield, enabling producers to evaluate these parameters effectively in their crops (El-Hendawy et al., 2022). Leaf Area Index (LAI) and Normalized Difference Vegetation Index (NDVI) have been widely used for assessing crop health and potential yield in various contexts (Belmahi et al., 2023). It is crucial to explore the underlying interrelationship for interpreting respective plant processes since they are key predictors in maize yield potential assessment in growth stages.

The current study indicates that the LAI values across growth stages have influenced crop yield. The LAI values during R0-R3 varied significantly across the years, being lowest in 2023 and highest in 2024, while the values in 2022 remained average between the two. This significantly influenced the crop yield irrespective of the cropping year conditions. The effects of the LAI might have partly influenced the highest yield in 2024 compared to other years, allowing maximum interception of the incoming radiation and maximizing the photosynthetic process (Raza et al., 2021). It brings a higher yield recorded in 2024 than in 2023, provided that 2023 received more precipitation than 2024. However, LAI values showing higher values in 2022 than in 2023 indicated the low yield in the 2022 to 2023 crop year. This could be partly influenced by the climatic conditions between the years that noted drought conditions in 2022, the wettest year 2023, and the moderate 2024. Further, the influence is brought by the genetic landscape of the hybrids as affected by the crop year in favor of the hybrid DKC4590 that indicated the highest LAI at VT in 2022 than other hybrids. This study investigated the relationship between NDVI and LAI of maize, examining their influence on yield and grain quality to enhance the understanding of crop monitoring for smart farming technologies. The findings imply that canopy reflectance data

could be an essential source for analyzing the ecological performance of plant growth dynamics and quality obtained in the field, thereby contributing to the development of smart farming technologies. Generally, the results obtained in this research are not substantially different from the existing results but confirm the importance of NDVI and LAI. The highest NDVI was recorded in the VT, followed by R0-R3. This aligns with Tamas et al. (2023), suggesting that earlier stages can predict yield, contrasting Yang et al. (2022), suggesting R3.

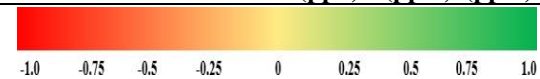
#### **4.2.3 Linkage between grain yield, fungal diseases development, and mycotoxin contamination on relative chlorophyll content and canopy reflectance parameters**

Correlation analysis significantly ( $P < 0.05$ ;  $p < 0.001$ ) determined the relationship between grain yield, various physiological parameters, fungi disease progression, and mycotoxin contamination (Table 11). In examining the effect of chlorophyll content on maize growth and yield, several studies found a significant positive correlation between these traits, which agrees with this study. The current research indicates a positive correlation between grain yield and SPAD, particularly at VT and R0-R3 ( $r = 0.69$  and  $0.76$ ). The contrary is the negative correlation at earlier stage V6 ( $r = -0.85$ ). Further, the current study indicated a positive correlation between LAI and NDVI at varying growth stages. LAI showed a positive correlation ( $r = 0.56$ ) with yield during early generative phases (R0-R3), while NDVI indicated a positive correlation during early vegetative growth V6 ( $r = 0.77$ ) and later vegetative tasseling VT ( $r = 0.70$ ).

On the other hand, the relationships between the examined physiological traits with fungi disease progression and mycotoxin contamination were significant ( $p < 0.05$ ;  $p < 0.001$ ). SPAD (R0-R3) indicated a relatively weak positive correlation with AF% ( $r = 0.79$ ) and FV% ( $r = 0.78$ ), and a relatively weak correlation ( $r = 0.46$ ) with AFB1 while demonstrating no correlation with mycotoxin FBs. On the contrary, it indicated a negative relationship with FG% ( $r = -0.62$ ) and DON ( $r = 0.47$ ). The exception was a significant negative strong correlation between LAI and (FG%, DON) during R0-R3 at ( $r = -0.87$ ,  $-0.83$ ) respectively.

Table 11: Implication of relative chlorophyll content, LAI, and NDVI on maize yield, fungal disease development, and mycotoxin contamination.

	Yield (kg ha <sup>-1</sup> )	SPAD -V6	SPAD -VT	SPAD -R0-R3	SPAD -R6	LAI -V6	LAI -VT	LAI -R0-R3	LAI -R6	NDVI -V6	NDVI -VT	NDVI -R0-R3	NDVI -R6	AF%	FV%	FG%	AFB1 (ppb)	FBs (ppm)	DON (ppm)
<i>Yield(kgha<sup>-1</sup>)</i>	1.00																		
SPAD-V6	-0.85**	1.00																	
SPAD-VT	0.69*	-0.34	1.00																
SPAD:R0-R3	0.76**	-0.67*	0.74	1.00															
SPAD-R6	-0.29	0.07	-0.30	0.21	1.00														
LAI-V6	-0.15	0.44	0.20	-0.37	-0.49*	1.00													
LAI-VT	-0.38	0.51*	-0.01	0.03	0.54*	0.25	1.00												
LAI:R0-R3	0.56*	-0.69*	0.24	0.79**	0.44	-0.74**	0.04	1.00											
LAI-R6	0.47*	-0.11	0.68*	0.39	-0.15	0.52*	0.32	-0.05	1.00										
NDVI-V6	0.77**	-0.77**	0.46*	0.85**	0.19	-0.50*	-0.06	0.86**	0.24	1.00									
NDVI-VT	0.70*	-0.34	0.73*	0.36	-0.56	0.54*	-0.08	-0.04	0.79**	0.31	1.00								
NDVI:R0-R3	0.41	-0.56*	0.19	0.43	0.41	-0.16	0.04	0.38	0.40	0.49*	0.18	1.00							
NDVI-R6	-0.38	0.20	-0.51	-0.35	0.39	0.06	0.52	-0.05	0.09	-0.09	-0.19	0.26	1.00						
AF%	0.82**	-0.68*	0.58*	0.79**	0.02	-0.31	-0.05	0.70*	0.40	0.85**	0.52*	0.31	-0.10	1.00					
FV%	0.81**	-0.69*	0.65*	0.78**	-0.04	-0.38	-0.26	0.63*	0.40	0.80**	0.49*	0.38	-0.20	0.89**	1.00				
FG%	-0.54*	0.65*	-0.14	-0.62*	-0.29	0.87**	0.28	-0.87**	0.20	-0.71**	0.12	-0.21	0.20	-0.61*	-0.64*	1.00			
AFB1(ppb)	0.04	-0.19	-0.09	0.46*	0.80**	-0.72**	0.32	0.72**	-0.14	0.51*	-0.39	0.25	0.30	0.43	0.40	-0.66*	1.00		
FBs(ppm)	0.35	-0.28	0.22	0.15	0.01	0.26	0.12	0.09	0.52*	0.21	0.43	0.57*	0.42	0.31	0.32	0.07	0.05	1.00	
DON(ppm)	-0.18	0.37	0.12	-0.47*	-0.60*	0.94**	-0.01	-0.83**	0.36	-0.56*	0.45	-0.15	0.00	-0.43	-0.40	0.90**	-0.83**	0.20	1.00



\*\*( $p < 0.001$ ); \*( $p < 0.05$ ), AF% = Proportion of kernels infected *A. flavus*, while FV% = Proportion affected by *F. verticillioides*. FG% = Proportion affected by *F. graminearum*. AFB1 = aflatoxin B1; FBs = fumonisin B1 + B2; DON = deoxynivalenol

On the contrary, LAI (R0-R3) indicated a positive correlation with AF% ( $r= 0.70$ , and  $0.35$ ) and FV% ( $r= 0.63$ ), and AFB1 ( $r= 0.72$ ) while no correlation was demonstrated between LAI and FBs. Another negative correlation was demonstrated between NDVI (V6) and FG% ( $r = -0.71$ ), DON ( $r = -0.56$ ), while at the same growth stage (V6), NDVI showed a positive correlation with AF% ( $r= 0.85$ ) and FV% ( $r = 0.80$ ) and AFB1 ( $r=0.51$ ) However, there was no distinctive correlation between NDVI and mycotoxin FBs.

It is well known that genotypes and mineral nutrition significantly affect maize yield (Bojtor et al., 2022). The current study showed a distinctive positive correlation between the chlorophyll content and yield during vegetative tasseling (VT) and early generative phases (R0-R3) stages. This observation may indicate that relative chlorophyll content during the vegetative tasseling (VT) and early generative phases (R0-R3) is essential and can be used to estimate the quantity of final maize production. The negative correlation with yield during the early V6 marks an important early warning indicator for nutrient management, N fertilization in particular, for heightening the index during the following stages to maximize assimilation and final production. It is because chlorophyll content can be increased by downstream elements promoting maize vegetative growth (Yang et al., 2023), signified by nitrogen dose significantly impacting relative chlorophyll content (SPAD) and other physiological traits.

While LAI and NDVI indicated an accelerative influence on AF% and FV% during R0-R3 and V6, respectively, on the contrary, the impact was beneficial concerning FG% and DON. The relativeness between AF% and FV% may depict the possibility of co-occurrence of the relative mycotoxins in agricultural production. This is backed by reported secondary infection relationships between AF and FV (Liverpool-Tasie et al., 2019), although this case did not show a defined relationship with the respective produced mycotoxins. The current results reflect a critical definitive correlation between the non-destructive indicators SPAD, NDVI, and LAI to final yield. However, their opposite direction influences fungi disease progression, and mycotoxin contamination of AF%, FV% vs FG%, and DON indicate an area of further concentration for optimum values to bring the balance for an informative inference.

### **4.3 The severity of ear rot and the levels of mycotoxins as influenced by nitrogen fertilization, hybrids used, and the process of inoculation**

Table 12 illustrates the effects of N dosage and hybrid susceptibility levels on the severity of ear rot and the production of mycotoxins. Across all evaluated years, it was observed that the application of nitrogen fertilization, the choice of hybrid, and the inoculation treatments significantly ( $p < 0.05$ ) affected the severity of ear rot and mycotoxin contamination levels.

With the *Fusarium* fungal diseases, nitrogen treatments affected FV%, indicating a significant increase of ear infections in 2022 and 2024 by 200% and 28%, respectively. However, in 2023, the infection was significantly higher at N90, and there were no differences between N0 and N150. Similarly, FBs production was significantly lowest at N0 and highest at N150 in 2022 and 2024. However, 2023 indicated highest significant ( $p < 0.05$ ) FBs production at N90 following  $N90 > N0 > N150$ . On the other hand, FG% indicated a substantial variation within years in 2022. The treatment at N150 showed the highest infection rate and lowest at the control. However, no significant variation was recorded within the years 2023 and 2024. Between years, a considerable infection was noted as influenced by nitrogen rate in the trend  $2023 > 2022 > 2024$ , with the lowest recorded in controls at 7.6%, 23.5%, and 0.61% and the highest recorded in N150 at 13.7%, 25.1%, and 0.94% in 2022, 2023 and 2024 respectively. The DON production indicated no differences with N rates within years and followed the same trend as FG % between years with N treatments. Although the percentage of aflatoxin (AF%) demonstrated statistical significance ( $p < 0.05$ ) across all observed years, the production of AFB1 showed significance ( $p < 0.05$ ) exclusively in the year 2022, not significant in 2024, and the infection did not produce any AFB1 in 2023.

The hybrid tolerance was significant ( $p < 0.05$ ), showing varying effects on fungal diseases and toxin production. FG% indicated a substantial decrease with hybrid tolerance in DKC4590 < GKT376 < P9610 in 2022 and 2023 and no significance in 2024. Additionally, DON production followed a similar trend. FV% and AF% indicated unpredictable trends in hybrids both within and between years. GKT376 indicated heightened FBs in all and AFB1 in 2022 and 2024, with no AFB1 recorded in all hybrids in 2023. Implementing inoculation treatments significantly impacted the percentage of ear rot severity. It resulted in the

production of mycotoxins, irrespective of the nitrogen rates and the tolerance levels of the hybrid, throughout all observed years. Both fungal infection and mycotoxin heightened in inoculated treatment compared to non-inoculated controls.

Table 12: N rates, hybrid selection, and ear inoculation influence the severity of ear rot and contamination of mycotoxin (Debrecen, 2022-2024)

Factors variation	for	The severity of ear rot			Mycotoxins Amounts		
		AF%	FV%	FG%	AFB1(ppb)	FBs(ppm)	DON(ppm)
<b>N application doses</b>							
2022	Ø	0.08	0.07	7.60	9.83	1.17	4.37
	N90	0.21	0.16	13.10	43.11	2.39	3.84
	N150	0.22	0.26	13.70	67.90	2.89	3.96
2023	Ø	0.15	0.17	23.50	< LOD	2.69	60.30
	N90	0.21	0.24	24.90	<LOD	3.57	59.00
	N150	0.18	0.16	25.10	<LOD	1.88	50.50
2024	Ø	0.36	0.46	0.61	32.46	1.34	<LOD
	N90	0.28	0.32	0.67	31.89	3.72	0.42
	N150	0.32	0.64	0.94	44.71	3.13	1.40
	<i>LSD</i> <sub>(0.05)</sub>	<b>0.11</b>	<b>0.13</b>	<b>5.59</b>	<b>33.49</b>	<b>1.26</b>	<b>26.96</b>
<b>Maize hybrid effects</b>							
2022	DKC4590	0.09	0.13	7.30	37.08	1.73	2.11
	GKT376	0.22	0.24	9.60	51.38	2.62	4.73
	P9610	0.19	0.11	17.50	32.38	2.11	5.34
2023	DKC4590	0.21	0.21	15.80	< LOD	2.51	37.10
	GKT376	0.17	0.22	27.70	<LOD	3.70	66.50
	P9610	0.16	0.14	29.90	<LOD	1.94	66.20
2024	DKC4590	0.37	0.62	0.69	37.22	2.90	0.25
	GKT376	0.31	0.35	0.85	38.10	3.00	1.62
	P9610	0.29	0.44	0.67	33.75	2.29	<LOD
	<i>LSD</i> <sub>(0.05)</sub>	<b>0.11</b>	<b>0.12</b>	<b>5.40</b>	<b>35.08</b>	<b>1.16</b>	<b>32.27</b>
<b>Inoculation effects</b>							
2022	Treated	0.28	0.33	22.90	62.59	3.02	8.11
	Untreated control	0.05	0.00	0.10	17.97	1.29	0.34
2023	Treated	0.32	0.33	48.80	<LOD	4.66	113.20
	Untreated control	0.03	0.05	0.10	< LOD	0.77	< LOD
2024	Treated	0.62	0.91	1.46	56.20	4.53	0.91
	Untreated control	0.02	0.04	0.01	16.50	0.94	0.36
	<i>LSD</i> <sub>(0.05)</sub>	<b>0.08</b>	<b>0.11</b>	<b>4.45</b>	<b>27.49</b>	<b>1.03</b>	<b>25.00</b>

Note: LOD - Limit of detection; AF = A. flavus; FV = F. verticillioides; FG = F. graminearum; AFB1 = aflatoxin B1; FBs = fumonisins B1+B2; DON = deoxynivalenol

#### 4.3.1 Nitrogen fertilization impacts on mycotoxin levels.

Nitrogen fertilization is a crucial factor influencing final maize yield and mycotoxin synthesis. The variability in toxin amounts observed at different N fertilizer doses used in this study underscores their associations' intricacies. While some studies suggest that higher nitrogen application rates lead to increased mycotoxin contamination, others present

conflicting findings. These inconsistencies emphasize the need to thoroughly explore how nitrogen application interacts with ear rot severity and mycotoxin production. The present study identified an overall rise in mycotoxin contamination, specifically for AFB1 and FBs, in 2022 as nitrogen levels increased. This outcome contradicts Blandino et al. (2008), which indicated that nitrogen deficiencies were associated with increased fumonisin contamination in maize.

Tubajika et al. (1999) observed a significant reduction in aflatoxin contamination, ranging from 34% - 45%, with the application of nitrogen fertilizer at rates between 50 - 250 kg ha<sup>-1</sup>, compared to plots without nitrogen (N0). The current study indicated a significant shift in 2023, as no AFB1 was detected, and a decrease in DON toxin contamination was correlated with higher nitrogen application when in solitary treatment. Interestingly, the highest levels of DON contamination were associated with the N0 treatment in 2023. In contrast, 2024 recorded the lowest DON levels, with negligible amounts found at N0 (Table 12). These results correspond with earlier findings, highlighting the complex associations between N application doses, the severity of ear rot, and the occurrence of mycotoxins in maize production (Madege et al., 2018; Simões et al., 2023; Krnjaja et al., 2021; Santos et al., 2023). Maize susceptibility to mycotoxin contamination is significantly affected by nitrogen application, indicating that such fertilization can both elevate and lower mycotoxin concentrations (Borràs-Vallverdú et al., 2022; Scarpino et al., 2022). Given the inconsistencies surrounding nitrogen fertilization outcomes, additional factors, including genetic profiles of hybrids and environmental conditions, may play a critical role in governing mycotoxin synthesis in maize following nitrogen usage. Evaluating the effects across a range of nitrogen applications is crucial, particularly because a substantial number of available research concentrates on higher nitrogen levels. This analysis could delve into a profound knowledge of the intricate dynamics between N application practices and mycotoxin synthesis, thereby supporting the establishment of specified agronomic practices to decrease mycotoxin risk in maize farming.

#### **4.3.2 Hybrid selection influences mycotoxin contamination in maize**

The findings indicate a substantial hybrid choice influence on mycotoxin contamination across all evaluated years. This observation aligns with Bocianowski et al. (2020), who reported that maize hybrids exhibiting superior tolerance demonstrate lower mycotoxin

contamination levels. In most cases, the hybrid DKC 4590, characterized by its lower susceptibility, consistently showed the least accumulation of mycotoxins. In contrast, GKT376 displayed higher levels of mycotoxin contamination. The hybrid P9610, which has an unspecified tolerance but is associated with greater yields, generally did not demonstrate a statistically significant difference from GKT376 for most mycotoxins, although it displayed the lowest levels of fumonisins (FBs) compared to other hybrids in 2023. Excluding this inconsistency, the overall results are consistent with findings from other research, reinforcing the suggestive recommendation that hybrids with good tolerance levels may alleviate the risks associated with mycotoxin contamination (Mesterházy et al., 2022; Szabó et al., 2018).

The inconsistencies in toxin production observed in hybrid P9610 to hybrids DKC4590 and GKT376, which have defined tolerance thresholds, align with the results reported by Barošević et al. (2022). Their finding highlighted inconsistencies and variable levels of mycotoxin contamination across maize hybrids linked to unidentified resistance capacities. These variations may result from the documented resistance traits of the hybrids assessed in this study. This situation emphasizes the critical significance of hybrid selection with well-documented resistance attributes for minimizing the potential risks of mycotoxin contamination. Notably, this study identifies a crucial insight: the susceptibility of hybrids to mycotoxin contamination is contingent upon both the specific type of mycotoxin and the inherent characteristics of the hybrid itself (Leite et al., 2021).

Furthermore, the performance of diverse maize hybrids significantly influenced the activity of mycotoxin-producing fungi, notably *A. flavus*, *F. verticillioides*, and *F. graminearum*. The results emphasize that the genetic characteristics of maize hybrids render different responses to toxigenic fungi colonization and their toxin synthesis capabilities. This variation indicates that efforts to enhance hybrid breeding to minimize mycotoxin contamination present considerable challenges. Although identifying maize hybrids that exhibit reduced susceptibility to fungal infections is crucial, the apparent disparities among hybrids suggest that many maize growers may prefer less vulnerable hybrids to such infections. This trend underscores the urgent requirement for targeted educational initiatives to guide farmers in recognizing the usefulness of choosing hybrids with established

resistance traits, thereby bolstering food safety and alleviating mycotoxin-related hazards in maize production systems.

#### 4.4 Nitrogen dosage and hybrid selection interactions affect ear rot severity and mycotoxin levels.

##### 4.4.1. Fungal ear rot diseases affected by N doses and hybrids selection

The various treatment interactions substantially impacted the severity of ear rot and mycotoxin contamination levels during the growing seasons of 2022 to 2024 (Table 13). In plots that were artificially inoculated, the effect of the treatments on ear rot severity was dynamic and statistically significant ( $p < 0.05$ ).

Table 13: Interactive effects of N rates and hybrid selection on the severity of ear and kernel rot (Debrecen, 2022-2024)

Year	N level	Hybrids	Artificial inoculation			Mean	Natural infected Control		Mean	
			AF%	FV%	FG%		Aspergillus (%)	Fusarium (%)		
2022	Ø	DKC4590	0.04	0.09	10.30	3.48	0.01	0.10	0.06	
		GKT376	0.15	0.22	9.60	3.32	0.00	0.00	0.00	
		P9610	0.21	0.12	25.60	8.64	0.05	0.00	0.03	
	N90	DKC4590	0.29	0.14	19.70	6.71	0.08	0.10	0.09	
		GKT376	0.34	0.57	19.50	6.80	0.09	0.11	0.10	
		P9610	0.24	0.21	39.00	13.15	0.21	0.10	0.16	
	N150	DKC4590	0.07	0.57	13.90	4.85	0.03	0.00	0.02	
		GKT376	0.77	0.66	28.20	9.88	0.01	0.10	0.06	
		P9610	0.43	0.34	40.10	13.62	0.01	0.20	0.11	
	<b>Mean</b>			<b>0.28</b>	<b>0.32</b>	<b>22.88</b>	<b>7.83</b>	<b>0.05</b>	<b>0.08</b>	<b>0.07</b>
	<i>LSD</i> <sub>(0.05)</sub>			0.24	0.16	10.29				
	2023	Ø	DKC4590	0.39	0.37	34.20	11.66	0.10	0.13	0.11
			GKT376	0.20	0.25	49.90	16.78	0.00	0.10	0.05
P9610			0.23	0.34	55.90	18.82	0.00	0.15	0.08	
N90		DKC4590	0.29	0.29	22.90	7.83	0.20	0.15	0.18	
		GKT376	0.40	0.56	59.00	19.99	0.00	0.00	0.00	
		P9610	0.39	0.27	67.30	22.65	0.00	0.00	0.00	
N150		DKC4590	0.26	0.25	37.10	12.54	0.00	0.05	0.03	
		GKT376	0.40	0.38	56.90	19.23	0.00	0.15	0.08	
		P9610	0.34	0.23	55.90	18.82	0.00	0.05	0.03	
<b>Mean</b>			<b>0.32</b>	<b>0.33</b>	<b>48.78</b>	<b>16.48</b>	<b>0.03</b>	<b>0.09</b>	<b>0.06</b>	
<i>LSD</i> <sub>(0.05)</sub>			0.17	0.17	13.23					
2024		Ø	DKC4590	0.80	1.19	1.46	1.15	0.03	0.05	0.04
			GKT376	0.48	0.67	1.02	0.72	0.04	0.00	0.10
	P9610		0.79	0.78	1.12	0.90	0.00	0.00	0.00	
	N90	DKC4590	0.59	0.69	0.98	0.76	0.00	0.01	0.04	
		GKT376	0.65	0.48	2.02	1.05	0.00	0.00	0.01	
		P9610	0.40	0.71	0.99	0.70	0.03	0.00	0.00	
	N150	DKC4590	0.71	1.62	1.62	1.32	0.09	0.02	0.15	
		GKT376	0.63	0.84	2.05	1.17	0.03	0.04	0.02	
		P9610	0.49	1.18	1.92	1.19	0.00	0.00	0.00	
	<b>Mean</b>			<b>0.62</b>	<b>0.91</b>	<b>1.46</b>	<b>1.00</b>	<b>0.02</b>	<b>0.01</b>	<b>0.04</b>
	<i>LSD</i> <sub>(0.05)</sub>			0.17	0.38	0.82				

AF% = Proportion of kernels infected by *A. flavus*, while FV% = Proportion affected by *F. verticillioides*.  
FG% = Proportion affected by *F. graminearum*.

In 2022 and 2023, AF % for GKT376 was higher than other hybrids, with an increase in N application. However, in 2024, AF % for DK4590 surpassed GKT376 at N150. Similarly, except in the N0 treatment, FV % was highest for the hybrid GKT376. On the contrary, FG % was highest for the hybrid P9610 in all N levels across years. The infection rate increased with N fertilizer.

The infection rates exhibited notable variation across 2022, 2023, and 2024. In 2022, the infection rates ranged between 9.6% and 40.1% for FG, 0.09% to 0.66% for FV, and 0.04% to 0.77% for AF. The subsequent year, 2023, demonstrated an increase in variability, with FG rates reaching 22.9% to 67.3%, FV showing 0.23% to 0.56%, and AF values 0.2% to 0.4%. By 2024, the infection rates for FG stood at 0.98% to 2.05%, FV from 0.48% to 1.62%, and AF from 0.4% to 0.8%. The mean infection rate values for AF, FV, and FG were recorded as 0.28%, 0.32%, and 22.88% for 2022; 0.32%, 0.33%, and 48.78% for 2023; and 0.62%, 0.915, and 1.46% for 2024 respectively. In the inoculated treatment plots, the FG ear rot severity was the most pronounced, showing an increase of 51% from 2022 to 2023. Conversely, the ear rot severity observed in un-inoculated natural infection controls was statistically not significant across varying N dosages and hybrids during the maize growing seasons from 2022 to 2024. Notably, the hybrid DKC4590, characterized by lower susceptibility, consistently exhibited the least FG ear rot severity across all N doses to P9610 and GKT376

#### **4.4.2 Influence of N Dosage and Hybrid Selection on Mycotoxin Synthesis**

Table (14) illustrates the combined influence of the N application dosage and genotypes of selected hybrid on mycotoxin contamination levels. In 2022, a notable interaction between the selected hybrids and nitrogen treatments affecting *A. flavus* mycotoxin contamination was observed, with statistical significance ( $p < 0.05$ ) identified across various fertilizer rates and hybrid types. The quantities of mycotoxins detected remained beneath the permissible contamination thresholds of 20 ppm for feeds and 4 ppm for food products as established by European Commission regulations (EC, 2006, 2007, 2002). The application of nitrogen fertilizers corresponded with an 89% increase in contamination, with the highest level recorded being 0.129 ppm for hybrid GKT376 at the nitrogen rate of N150, in contrast to the lowest level of 0.014 ppm for hybrid P9610 at N0 during 2022. Notably, no aflatoxin

was quantified in either inoculated or non-inoculated plots during 2023. In 2022, the highest AFB1 in the inoculated plots was lower at the N0 treatment and increased with the N rate, with GKT376 recording the heightened amounts. In 2024, the amount of aflatoxin B1 recorded was insignificant and lower than in 2022; however, the average infection was lower in 2022 at 0.28% compared to 0.62% in 2024. Conversely, in the non-inoculated treatment, the amount of AFB1 produced in 2022 and 2024 indicated the contrary.

Table 14: Interaction effects of N doses and hybrid genetics on mycotoxin contamination across years (Debrecen, 2022-2024).

	N Level	Hybrids	Artificial inoculated			Untreated Control			
			DON(ppm)	FBs(ppm)	AFB1(ppb)	DON(ppm)	FBs(ppm)	AFB1(ppb)	
2022	Ø	DKC4590	4.11	0.97	19.95	<LOD	0.47	4.44	
		GKT376	9.78	2.49	15.94	<LOD	0.93	4.23	
		P9610	12.33	1.71	14.41	<LOD	0.48	<LOD	
	N90	DKC4590	5.82	3.06	26.68	<LOD	2.34	14.21	
		GKT376	8.98	3.90	124.20	<LOD	1.48	0.88	
		P9610	8.21	2.26	29.99	<LOD	1.33	62.67	
	N150	DKC4590	2.71	2.37	119.40	<LOD	1.15	37.80	
		GKT376	9.60	6.59	128.56	3.10	0.33	34.46	
		P9610	11.48	3.81	84.20	<LOD	3.09	3.00	
	<b>Mean</b>			<b>8.11</b>	<b>3.02</b>	<b>62.59</b>	<b>3.1</b>	<b>1.29</b>	<b>20.21</b>
	<i>LSD(0.05)</i>			4.60	1.69	99.86	*	1.69	99.86
	2023	Ø	DKC4590	54.40	5.34	<LOD	<LOD	1.09	<LOD
GKT376			154.60	5.19	<LOD	<LOD	0.66	<LOD	
P9610			152.90	3.87	<LOD	<LOD	<LOD	<LOD	
N90		DKC4590	91.40	4.33	<LOD	<LOD	1.99	<LOD	
		GKT376	120.90	8.81	<LOD	<LOD	1.44	<LOD	
		P9610	141.50	4.86	<LOD	<LOD	<LOD	<LOD	
N150		DKC4590	76.60	2.32	<LOD	<LOD	<LOD	<LOD	
		GKT376	123.90	4.60	<LOD	<LOD	1.47	<LOD	
		P9610	102.40	2.59	<LOD	<LOD	0.30	<LOD	
<b>Mean</b>			<b>113.17</b>	<b>4.66</b>	*	*	<b>1.16</b>	*	
<i>LSD(0.05)</i>			76.29	1.717	*	*	*		
2024		Ø	DKC4590	<LOD	2.70	41.52	<LOD	<LOD	29.98
	GKT376		<LOD	2.72	53.80	<LOD	<LOD	8.27	
	P9610		<LOD	2.63	52.97	<LOD	<LOD	8.22	
	N90	DKC4590	<LOD	4.95	38.67	0.93	4.64	13.87	
		GKT376	<LOD	7.93	68.31	1.56	0.90	<LOD	
		P9610	<LOD	3.67	70.50	<LOD	0.26	<LOD	
	N150	DKC4590	0.60	4.26	57.75	<LOD	0.83	41.51	
		GKT376	7.25	4.78	61.80	0.56	1.69	36.4	
		P9610	<LOD	7.11	60.51	<LOD	<LOD	10.27	
	<b>Mean</b>			<b>3.93</b>	<b>4.53</b>	<b>56.20</b>	<b>1.02</b>	<b>1.67</b>	<b>20.04</b>
	<i>LSD(0.05)</i>			3.20	3.26	24.86	3.20	3.26	24.86

Note: AFB1 = aflatoxin B1; FBs = fumonisin B1+B2; DON = deoxynivalenol; LOD - Limit of detection.

The fusarium mycotoxins, DON, and FBs significantly differed ( $p<0.05$ ) between all nitrogen rates and hybrids used in the three years. DON contamination in artificially inoculated plots followed the same trend in % ear rot severity for FG, which was higher in

2023, while in 2024, no detectable deoxynivalenol (DON) levels were detected during the lowest infection rate FG% except at N150 for DKC4590 (0.6 ppm) and GKT376 (7.25 ppm). Data from 2022 and 2023 indicate that the hybrid DKC4590 consistently exhibited the lowest DON levels compared to other hybrids, showing statistically significant differences ( $p < 0.05$ ). The ranking of DON levels in 2022 followed the order P9610>GKT376>DKC4590, whereas in 2023, the order was GKT376>P9610>DKC4590. In natural conditions, DON synthesis was minimal, with its presence noted solely for the hybrid GKT376 at a nitrogen rate of N150 in 2022; however, no DON was detected across all hybrids and nitrogen rates in 2023. In contrast, the production of Fusarium mycotoxins (FBs) in inoculated maize showed significant variation ( $p < 0.05$ ) across hybrids and nitrogen rates in both years. Significant differences were noted within specific nitrogen rates at N150 in 2022 and at both N90 and N150 in 2023. Across all observed years, the highest levels of FBs were recorded for the hybrid GKT376, measuring 6.59 ppm at N150 in 2022 and peaking at 8.8 ppm at N90 in 2023. The result on GKT376 indicates a consistent increase in the production of Fusarium toxins (FBs) across all nitrogen (N) application rates between 2022 and 2023. However, in 2024, at N150, P9610 exhibited the highest concentration of FBs at 7.11 ppm), followed by GKT376 at 4.78 ppm, while DKC4590 recorded the lowest level at 4.26 ppm. Throughout 2022 and 2023, DKC4590 consistently demonstrated the lowest FB levels across various N rates, although no significant differences were observed among the hybrids during these years. In 2024, P9610 registered the lowest FB levels, but these were not statistically significant compared to DKC4590. Furthermore, in the non-inoculated control group for the year 2022, a significant variation ( $p < 0.05$ ) in FBs among the hybrids at the N150 rate was noted, while in 2023, FBs levels across all hybrids remained statistically insignificant across all nitrogen application rates.

#### **4.4.3. Mycotoxin contamination levels in maize kernels: evaluating nitrogen application dosage and hybrid varieties interactions**

The findings indicate that applying nitrogen affects the intricate patterns of individual mycotoxin levels, which are significantly influenced by variations associated with the specific maize hybrid used. While there is a noticeable interaction between the rate of nitrogen application and the different maize hybrids concerning shifts in mycotoxin concentrations in the kernels, this relationship exhibits variability. Specifically, an additive

effect is observed in the deoxynivalenol (DON) contamination trend relative to the nitrogen fertilization rate, which is further complicated by the differing responses of the various hybrids.

The hybrid with a higher tolerance level to infection and mycotoxin contamination (DKC4590) showed less contamination than the less tolerant hybrid GKT376 and the hybrid P9610 in 2022 and 2023. DON indicated the lowest record in 2024 with a negligible amount and insignificant between DKC4590 and P9610, whereas GKT376 recorded the highest at 7.25. The findings reveal a significant trend in nitrogen rate performance, with the medium nitrogen application (N90) exhibiting superior outcomes in 2022 compared to 2023. Notably, GKT376 and the hybrid P9610 displayed no significant differences across all nitrogen treatments, implying that the hybrid's genetic attributes dominate regardless of nitrogen levels. The effects of the interaction between the accumulation of FBs and nitrogen fertilization and hybrid types remain uncertain. In 2022, elevated levels of FBs were noted at a nitrogen rate of N150, contrasting with 2023, where the control treatment (N0) showed markedly higher FBs concentrations and the highest rate (N150) yielded lower levels. Furthermore, the FBs metrics for 2024 aligned closely with those recorded in 2022 at the N90 treatment. This inconsistency underscores that manipulating nitrogen rates and hybrid varieties may lead to fluctuating mycotoxin levels across diverse maize cultivars, potentially establishing conditions associated with high and low mycotoxin risks.

The results indicate that the average incidence of *Aspergillus* ear rot increased from 0.28% in 2022 to 0.32% in 2023, reaching its peak at 0.62% in 2024. However, AFB1 contamination was only recorded in the years 2022 and 2024, with a notable contrast in levels; specifically, the year 2024 exhibited a considerably elevated AFB1 concentration of 128.56 ppb at the N150 level, in contrast to the lowest recorded level at N0. The amount recorded in 2024 was insignificant within and between N rates and relatively lower than in 2022. The visual evaluation of AF% (the mere presence of fungal mycelium) is an inadequate indicator of the quantity of aflatoxin produced. This assertion is supported by data from treatments inoculated with *A. flavus*, which exhibited an average ear rot percentage ranging from 0.05% to 0.28% in 2022. Despite these low ear rot levels, the levels of AFB1 toxin in hybrids were higher than the AF% values recorded, specifically 0.03% to

0.32% and 0.02% to 0.62% in 2023 and 2024, respectively, in both natural and inoculated treatments, which notably produced no AFB1 in comparison to 2022. This phenomenon may be attributed to the variations in weather patterns between the growing seasons. The present study agrees with the findings of Scarpino et al. (2018), highlighting that the production of mycotoxins results from complex metabolic mechanisms driven by fungal adaptation to diverse environmental and climatic factors as well as various stressors. Giorni et al. (2019) elaborate on these stressors and conditions, noting that aspects such as nutrient availability, temperature, moisture levels, water activity, relative humidity, substrate composition, and the application of fungicides play significant roles in determining the species of fungi that proliferate and, consequently, the levels of mycotoxins in crops. In the current investigation, specific attention is given to the impact of rainfall (which influences moisture content) and humidity on the severity of ear rot and the mycotoxin contamination from *A. flavus* and *F. graminearum*. These factors exhibited substantial variability during the growing seasons from 2022 to 2024, as depicted in Figure 5 and summarized in Table 5.

In artificial inoculation, treatments provided a reduction in contamination due to the interaction between nitrogen fertilization and hybrids, indicating the improved performance of DKC4590 and a moderate N rate of 90 kg ha<sup>-1</sup>. The level of recorded mycotoxins, specifically DON, was above the European maximum tolerable levels (MTL). However, under natural conditions, the levels of Fusarium mycotoxins DON and FBs recorded were below the European MTL for unprocessed maize at 1.75 ppm, 4 ppm for food, and 20 ppm for feed, promising the integration to help manage toxins in natural conditions.

#### **4.4.4 Analysis of the correlation between ear rot and kernel damage and the impact on mycotoxin production**

Regression and correlation analyses were conducted to assess mycotoxin production due to nitrogen's effect on ear rot severity in selected hybrids (Figure 14 and Table 15). Pooled yearly general means facilitated regression analysis and two-tailed Pearson correlation to explore ear rot severity relations among fungal isolates and nitrogen treatments. This methodology evaluated the relationship between ear rot severity, fungal isolates associated with the selected hybrids, and differing nitrogen treatment levels.

All isolates exhibited varying degrees of correlation between the severity of ear rot and the corresponding mycotoxin production in the examined fungal species. Specifically, the correlation between AF% and AFB1 synthesis was assessed, recording a weak and not significant ( $p>0.05$ ) positive relationship ( $r = 0.43$ ). Conversely, FV% and FBs production revealed a weak positive, not significant relationship ( $r = 0.32$ ). Notably, FG% ear rot severity demonstrated a strong positive relationship with DON production, characterized by a correlation coefficient of  $r = 0.90$ , which was statistically significant at  $p<0.001$ . Additionally, a negative correlation of  $r = -0.66$  ( $p<0.05$ ) was observed between FG% ear rot severity and AFB, indicating an inverse relationship, while no correlation was established between FG% and FBs contamination. Furthermore, AF%, FBs synthesis, FV%, and AFB1 production all exhibited weak nonsignificant positive correlations.

*Table 15: Pearson correlation coefficients for ear rot and mycotoxin levels (Debrecen, 2022-2024).*

	AF%	FV%	FG%	AFB1(ppb)	FBs(ppm)	DON(ppm)
AF%	1.00					
FV%	0.89**	1.00				
FG%	-0.61*	-0.64*	1.00			
AFB1(ppb)	0.43	0.40	-0.66*	1.00		
FBs(ppm)	0.31	0.32	0.07	0.05	1.00	
DON(ppm)	-0.43	-0.40	0.90**	-0.83**	0.20	1.00

\*\*( $p<0.001$ ); \*( $p<0.05$ ), AF% = Proportion of kernels infected by *A. flavus*, while FV% = Proportion affected by *F. verticillioides*. FG% = Proportion affected by *F. graminearum*. Color coding indicates correlations: green indicates a positive correlation, yellow signifies no correlation, and red denotes a negative correlation.

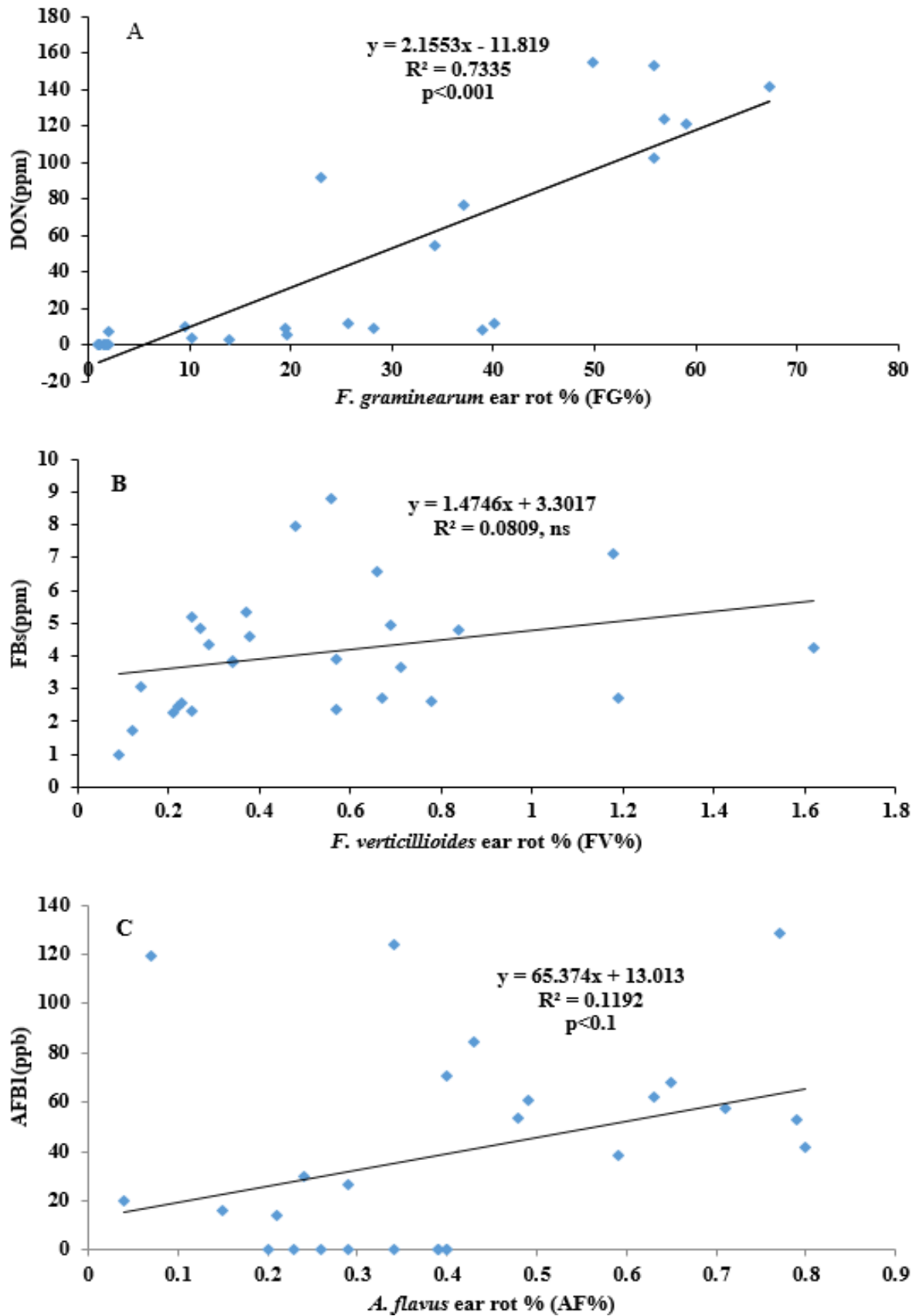


Figure 14: Analyzing the relationships between the severity of ear rot and the production of mycotoxins reveals a regression relationship: (A) FG% vs. DON; (B) FV% vs. FBs; (C) AF% vs. AFBI

Mycotoxins directly correlate with fungal infections, as Giorni et al. (2019) demonstrated. This observation aligns perfectly with the current research findings, explaining that the severity of each respective fungal infection showed a strong positive correlation to the levels of toxins produced (Figure 14). Consequently, the results also indicate a concerning trend regarding the co-occurrence of the Aspergillus toxin AFB1 and FBs produced by *F. verticillioides* (Table 15). The findings presented by Casu et al. (2024) reinforce the concerns regarding co-contamination of mycotoxins in the agricultural sector. The concurrent infection of agricultural plants by various mycotoxigenic fungal strains suggests that agricultural commodities are frequently subjected to co-contamination, which is particularly alarming. The capability of these fungal strains to produce multiple mycotoxins concurrently due to potential synergistic interactions among diverse toxins exacerbates the issue. As such, a thorough investigation into the environmental factors becomes essential to elucidate the additive effects of these various elements that influence the concentration of mycotoxins in agricultural products. This understanding is vital for assessing food safety and public health implications.

#### **4.5 Principal component analysis: general associations of the examined predictors on yield, physiological parameters, fungal disease development, and mycotoxin contamination**

The principal component analysis investigated the associations between grain yield, different physiological parameters, fungal disease development, and mycotoxin contamination as affected by selected maize hybrids and N rates in the three growing years. The variability in cropping year, N fertilization, and hybrids resulted in notable shifts in the named examined variables.

The intricate relationships between fluctuations in weather conditions during growing seasons, crop yield, quality metrics, fungal disease incidence, and mycotoxin levels were analyzed using principal component analysis (PCA), as illustrated in Figure 15. The results of the PCA revealed a significant correlation between the crop year, grain yield (GY), the development of fungal diseases, and mycotoxin contamination levels. Principal Component 1 (PC1), which accounts for 33.26% of the observed variability, demonstrated a notable association between quality parameters such as protein and starch content and AFB1, specifically related to the crop year 2022. Additionally, PC1 indicates pronounced

influences on yield metrics, including AF%, FV%, and FB production in 2024. Conversely, Principal Component 2 (PCA2), which explains 27.39% of the variability, highlights substantial year effects on DON levels and FG% observed in 2023.

The PCA strongly highlights the significant variations in weather conditions experienced during each cropping year, which affected the examined parameters. Principal Component Analysis (PCA) elucidates the complex relationships among the various parameters under consideration by analyzing the pertinent weather conditions for each cropping year. PCA groups the predictor variables that exert the most substantial influence on the assessed variables into clusters. These clustered patterns illustrate the impacts of the evaluated factor(s) on the corresponding variables, facilitating informed inferences regarding the trends of the affected variables, provided that the underlying characteristics of the predictor factors and their potential effects on the dependent variable are understood.

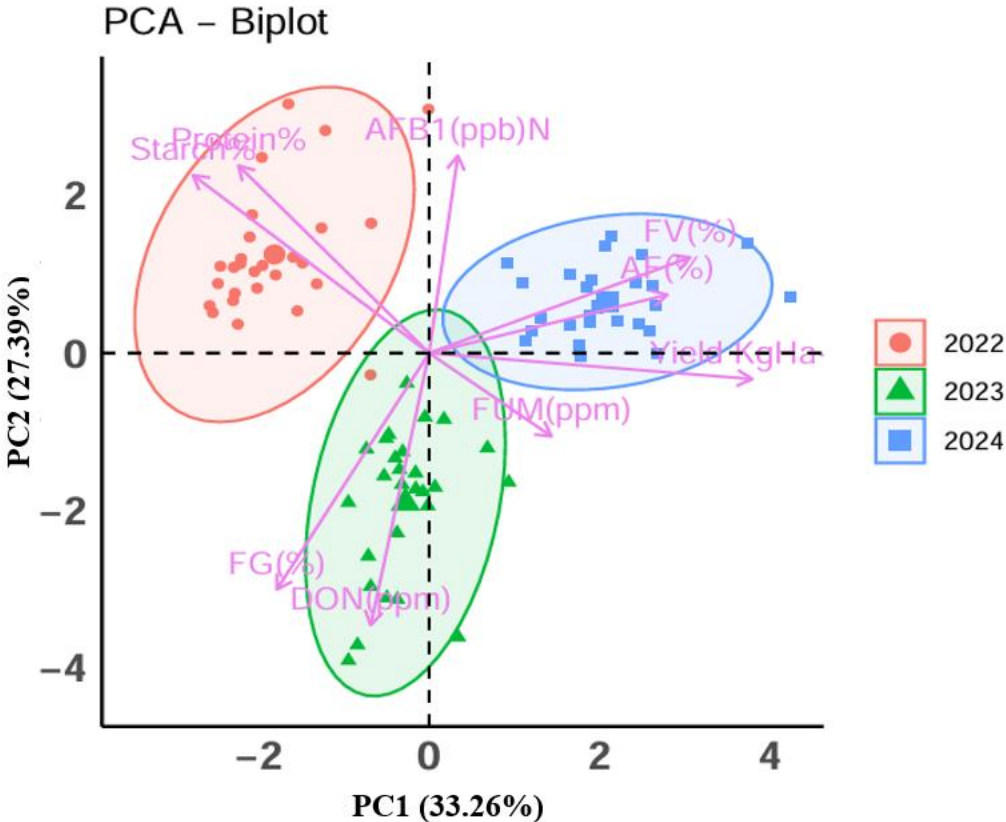


Figure 15: The principal component analysis biplot showing the relationships of crop year on yield, fungal disease development, and mycotoxin contamination. The first principal component explains 33.26% of the variance, while the second principal component contributes 27.39%, making a total variability of 60.65%

The analysis of the complex interplay between weather variability and cropping years reveals significant patterns in the PCA biplot, which illustrates distinct clusters corresponding to shifts in various variables throughout the cropping year (Figure 16). Notably, protein content, starch, and AFB1 variability strongly correlated with 2022. This year was characterized by drought conditions, marked by the lowest precipitation levels, resulting in reduced grain yields, elevated protein content, and decreased starch levels. Conversely, the production of AFB1 increased due to conducive weather conditions; however, the infection rate of *A. flavus* (AF %) remained comparatively low and was not influenced by the 2024 cluster.

In terms of grain yield, a strong correlation was observed with the year 2024, aligned with PC1, which indicated the highest yield levels, surpassing those of 2022 and 2023. This increase is closely linked with physiological parameters such as the SPAD index during the vegetative tasseling (VT) stage, along with the Leaf Area Index at VT (LAI-VT) and Normalized Difference Vegetation Index (NDVI). These relationships underscore the complex influence of environmental conditions on yield, suggesting that year-specific weather patterns profoundly affect the interplay of various variables.

Additional patterns indicate that 2023 significantly impacted the production of Fusarium mycotoxins, specifically Deoxynivalenol (DON), and the relative ear rot infection caused by *F. graminearum* (FG%). Unlike the relationship observed between AFB1 and AF%, higher levels of FG% were strongly associated with increased DON production. It suggests that the infection rate is a predictive indicator for the quantities of DON mycotoxin generated. Consequently, the effects of each year on the principal components PC1 and PC2 highlight the influences of grain quality and AFB1 in 2022, yield and physiological parameters in 2024, and Fusarium ear rot along with DON in 2023. The cropping season 2024 exhibited minimal variability in its impact on the affected traits, as evidenced by smaller ellipse on their positive positioning along PC1. In contrast, the broader dispersion illustrated by the ellipses for the years 2022 and 2023 across both PC1 and PC2 indicates a wider degree of variability in the influences on traits such as protein content, starch, AFB1, DON and NDVI during the vegetative tasseling (VT) growth stage. Furthermore, the directional magnitude of the arrows' correspondence reinforces this variability assessment.

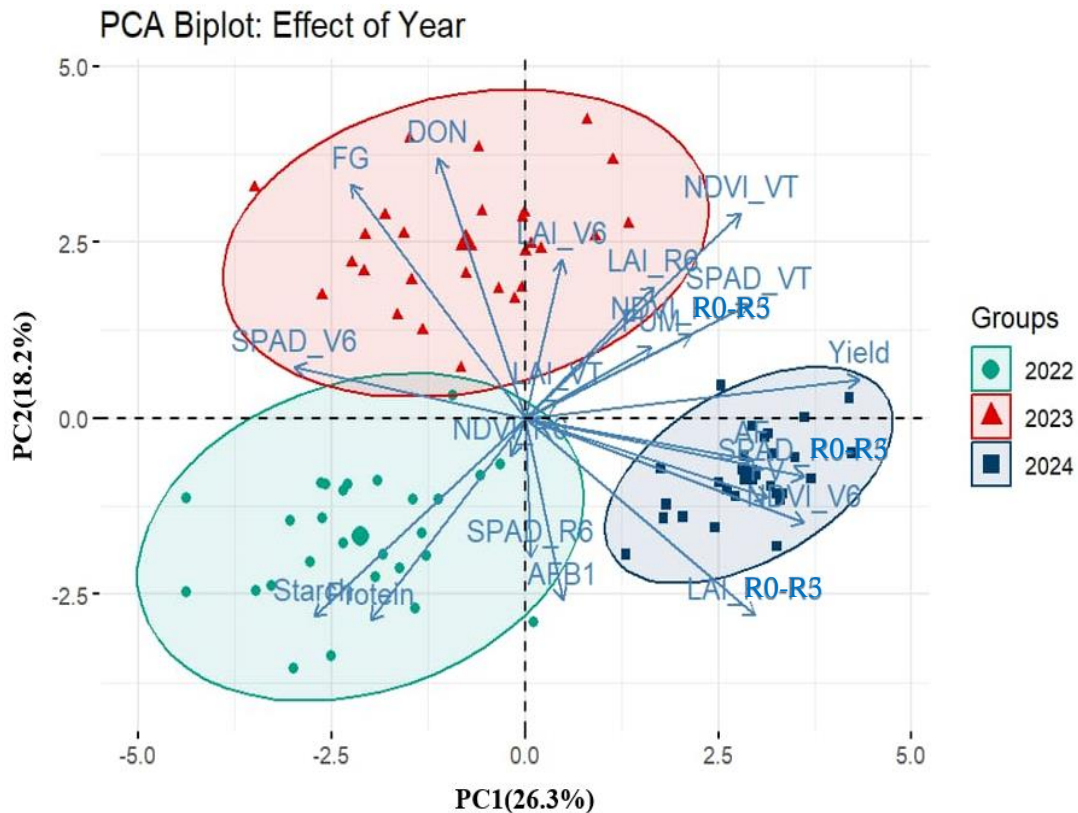


Figure 16: The principal component analysis biplot showing the relationships of crop year on yield, SPAD, NDVI, LAI, fungal disease development, and mycotoxin contamination. The first principal component explains 28% of the variance, while the second principal component accounts for 16.05%, making a total variability of about 44.05%. Clustering patterns of observation, direction, and magnitude of variables contributing to variability are presented by ellipses and arrows (Debrecen, 2022-2024).

Another principal component, biplot, illustrates the significant effects of nitrogen fertilization on grain yield, various physiological traits, the progression of different fungal diseases, and the consequent production of mycotoxins (Figure 17). This biplot reveals that principal component 1 (PC1) contributes to 26.3% of the total variability of the N rates influence, while principal component 2 (PC2) accounts for 18.2%, making a total variability of 44.5%

The PC biplot indicates that the Ø (no nitrogen) treatment demonstrates the most variability between the other N rates stretching further toward the left between PC1 and PC2. The N150 and N90 groups overlap much more closely. This overlapping suggests that these groups exhibit similar trait responses at moderate and high nitrogen levels. However, N90 is more centralized between N0 and N150, with the latter stretching toward the right between PC1 and PC2. The positioning depicts N90 as likely stable on the influence of the

examined variables. Additionally, the specific positioning of the traits within the biplot suggests that nitrogen application notably affects key quality attributes such as starch and protein content in the maize plants. Furthermore, the spread of data points observed within each treatment group indicates the degree of variability in the maize response to different levels of nitrogen application, highlighting essential insights regarding how maize might react to varying nitrogen inputs in agricultural practices.

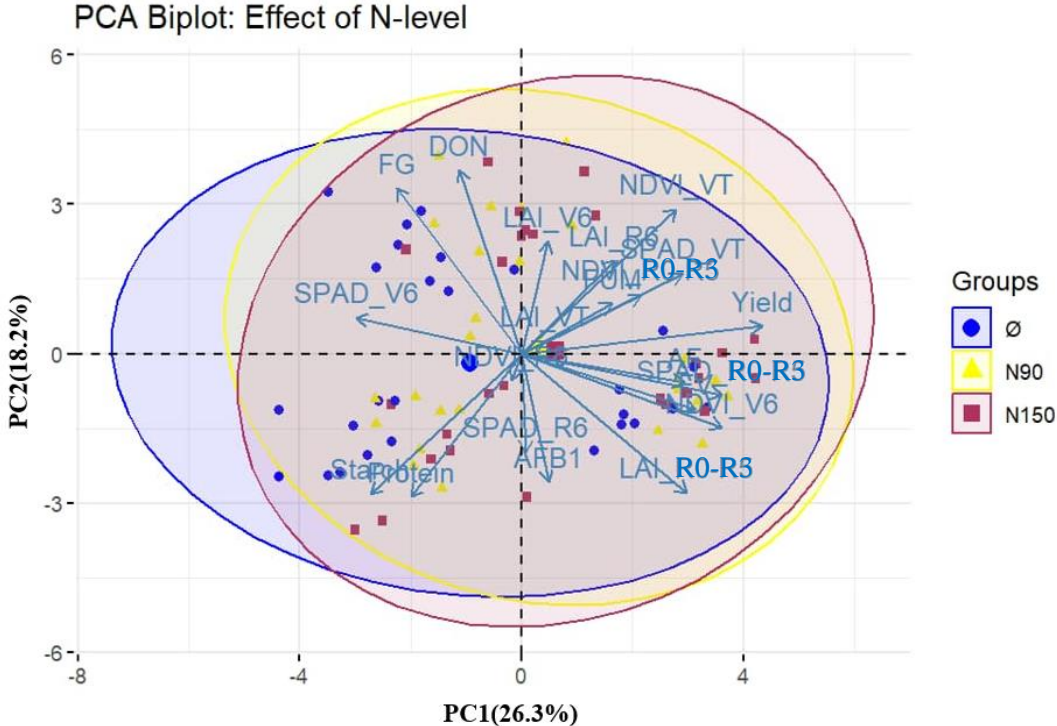


Figure 17: Effects of nitrogen fertilization on grain yield, quality, physiological traits, fungal disease development, and mycotoxin contaminations of maize (Debrecen, 2022-2024).

The fourth PC biplot indicates the influence of the genetic landscape of the selected hybrids on the examined variables, such as physiological parameters, grain yield and quality traits, fungal disease development, and mycotoxin contamination (Figure 18).

The overlapping ellipses suggest a similarity among the genotypes rather than the presence of distinct groups. Nonetheless, the PCA biplots reveal that hybrids P9610 and GKT376 exhibit greater variability concerning the assessed variables, implying a lack of stability and consistency in these hybrids. It is further evidenced by the broader dispersion observed along the principal components. Additionally, the variance between these hybrids is oriented in opposing directions relative to the principal components. Hybrid P9610 stretches

on the negative side of PC1, whereas GKT376 demonstrates a broader distribution across both PC1 and PC2.

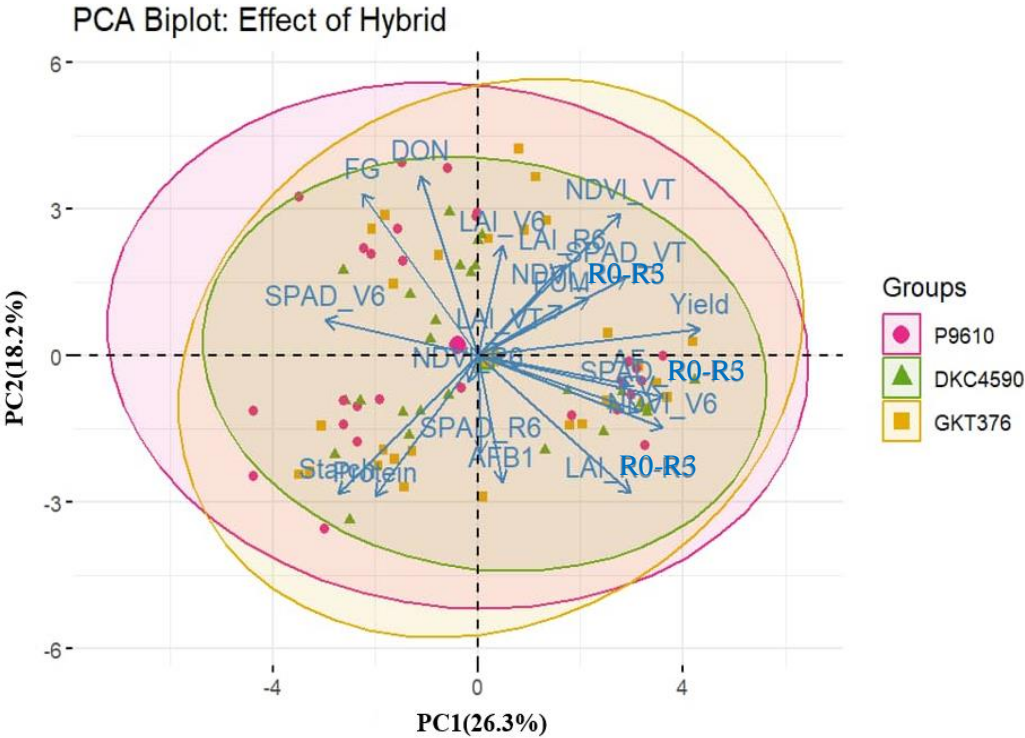


Figure 18: Effects of selected hybrid on grain yield, quality, physiological traits, fungal disease development, and mycotoxin contaminations (Debrecen, 2022-2024)

In contrast, a smaller ellipse and a more centralized distribution along the principal components characterize hybrid DKC4590. This pattern suggests reduced variability regarding the significant effects on the evaluated traits, indicating that DKC4590 possesses more excellent stability and consistency in its influence on the examined traits.

## **5. CONCLUSIONS AND RECOMMENDATIONS**

### **5.1 Annual weather variability, N fertilization, and hybrid selection on yield**

Under varying weather conditions between crop cultivation years, this study evaluated the effects of nitrogen fertilization and selected maize hybrids on yield and mycotoxin contaminations. The study further assessed the usefulness of physiological traits measured non destructively, which can be used to give inferences on potential shifts of the central themes. There was a general change in overall yield across the years. The change indicated the lowest yield in the year that received the least precipitation. Similarly, the year with the least precipitation was associated with reduced relative chlorophyll content (SPAD), LAI, and canopy reflectance indices of NDVI.

Following the years' trend, nitrogen fertilization did not indicate a significant influence on yield between the moderate and higher nitrogen rates during the dry year. A substantial variation in yield between hybrids was recorded in the interaction of hybrids with N rates. The hybrid DKC4590 yielded higher yields, which were recorded at a moderate N rate of N90 than at N150. The hybrid DKC4590 demonstrated superior performance during the year characterized by lower precipitation in 2022, achieving a statistically significant yield of 9.1 tons per hectare. In contrast, 2023 did not showcase any notable differences among the hybrids at nitrogen rates of N90 and N150, with GKT376 recording the lowest yield at N0. In 2024, DKC4590 excelled again, yielding 22.4 tons per hectare at N90, while GKT376 yielded 22.5 tons per hectare at N150 while revealing no significant yield differences between the two hybrids at the moderate and higher rates.

Therefore, this study advocates for DKC4590 as the top-performing hybrid across varying N rates and recommends the moderate N rate of N90 to enhance hybrid performance during dry and wet growing seasons. The crop year emerged as a crucial determinant of nitrogen (N) rates and hybrid performance in terms of grain yield. However, hybrids exhibited varied responses to different N rates concerning yield outcomes.

### **5.2 Yearly weather conditions and mycotoxin contaminations**

Meteorological conditions play a crucial role in colonization, disease development, and mycotoxin synthesis. These conditions can be dry and warm, moderately rainy and warm,

or include higher precipitation with mild temperatures, impacting these processes uniquely. AF% and FV% by *A. flavus* and *F. verticillioides* were consistent in 2022 and 2023, with notable infections in 2024. FBs from *F. verticillioides* were not significant and under European maximum tolerable levels. AFB1 production by *A. flavus* was absent in the humid 2023, with the highest levels found in the dry 2022, attributable to warmer, drier conditions favoring *A. flavus* and mycotoxin contamination. In contrast, *F. graminearum*'s FG% peaked during the wet 2023 year, resulting in increased deoxynivalenol (DON) production. Despite increased FV% in 2024, no significant changes in fumonisin production were observed, indicating that weather variability didn't impact FBs.

### **5.3 hybrid selection and N fertilization on mycotoxin contamination**

The results demonstrate a significant impact of hybrid selection on mycotoxin contamination over the years assessed. In most cases, the hybrid DKC 4590, characterized by its lower susceptibility, consistently showed the least accumulation of mycotoxins than P9610 and GKT376. These variations may stem from the resistance traits of the hybrids studied. This underscores the importance of selecting hybrids with well-documented resistance attributes to mitigate mycotoxin contamination risks. The results highlight that maize hybrids display varying genetic characteristics that influence their response to toxigenic fungi and toxin production. This variation poses challenges for breeding hybrids to reduce mycotoxin contamination.

Identifying less susceptible hybrids is essential, yet differences among them suggest that growers might lean toward those less prone to infections. This trend points to an urgent need for educational initiatives to help farmers understand the benefits of selecting hybrids with resistance traits, enhancing food safety, and reducing mycotoxin risks in maize production. Nitrogen fertilization significantly affects maize yield and mycotoxin production. The varying toxin levels observed with different N fertilizer doses highlight complex associations. Mycotoxins AFB1 and FBs increased with N in 2022, while DON decreased with N in 2023 between the control (N0) and other N rates, respectively. Thus, the individual application of nitrogen reveals a complex relationship involving various mycotoxin contaminations, showcasing contrasting effects at different nitrogen rates. The interaction with selected hybrids shifts the sole N trends following the hybrids'

susceptibility levels with the less susceptible hybrid DKC4590 demonstrating reduction of contamination at moderate N rate N90.

The overall contamination of mycotoxins for the experiment seasons for AFB1, DON and FBs at N90 and N150 was; DKC4590, (21.8, 59.1 ppb), (32.4, 26.6ppm) and 4.1, 3.0 ppm); P9610, (33.5, 48.2 ppb), (49.6, 38.0 ppm) and (3.6, 4.5 ppm); GKT376, (64.2, 63.5 ppb), (43.3, 46.9 ppm) and (6.9, 5.3 ppm) respectively. The hybrid DKC4590 indicated lower contaminations of mycotoxins for all fungi isolates of FG, FV, and AF at N90, suggesting that proper hybrid selection can reverse sole nitrogen application complexity at a moderate rate of 90 kg $\text{ha}^{-1}$ .

#### **5.4 SPAD, LAI, and NDVI implication on yield and mycotoxin contamination**

The indices SPAD, LAI, and NDVI measured at the vegetative stage (VT) and the reproductive stages R0-R3 exhibit a strong and positive correlation with the overall crop yield. Thus, when suitable and appropriate hybrids are chosen carefully, these indices can effectively function as reliable indicators for predicting crop yield at the VT and the R0-R3 stages of growth. This predictive capability highlights the importance of monitoring these indices to optimize yield outcomes in agricultural practices.

## 6. NEW SCIENTIFIC RESULTS

1. The hybrid DKC4590 at moderate N90 increased the overall yield by 7.2% than at N150 surpassing P9610 and GKT376 with no significant differences between all hybrids at N150. It recorded the overall yield of 14.8 tonsha<sup>-1</sup> vs. 13.8 and 14 tonsha<sup>-1</sup> for P9610, and GKT376 at N90 while recording 13.8 tonsha<sup>-1</sup> vs. 13.86 and 13.9 tonsha<sup>-1</sup> for P9610 and GKT376 at N150 respectively.
2. Mycotoxins AFB1 and FBs increased with N by (79% and 85.5%, 51% and 59.5%) in 2022, while DON decreased with N by 2% and 19.4 % in 2023 between the control (N0) and other N rates, respectively. Thus, the individual application of nitrogen reveals a complex relationship involving various mycotoxin contaminations, highlighting contrasting effects at different nitrogen rates.
3. At N90, hybrid DKC4590 exhibits a reduction in contamination of AFB1, DON and FBs by 66%, 35% and 25% respectively in comparison to the GKT376 hybrid, while recording a decrease of 35% and 19% AFB1 and DON against P9610 hybrid. Thus, when paired with a moderate nitrogen application rate of N90, the strategic selection of hybrids results in a substantial decrease in the risk of mycotoxin contamination. Notably, no clear trend in contamination risk was observed at the higher nitrogen rate of N150.
4. Fungal disease infection rate vs. mycotoxin contamination as affected by the interactions of hybrids and N rates is strongly suggestive regarding FG% and DON production. On the contrary, the favorability of the weather conditions of the cropping season for AF% and AFB mycotoxin contamination is a significant consideration. AF% indicated 0.28%, 0.32%, and 0.62% in 2022, 2023, and 2024 respectively, while 2022 showed the most heightened AFB1 levels.
5. The SPAD, LAI, and NDVI indices measured at the vegetative stage (VT) and reproductive phases (R0-R3) exhibited a positive correlation with crop yield; however, the highest average indexes record was at VT (58.94) in the range between 54.82 to 62.31 for SPAD, (0.76), between 0.68 to 0.81 for NDVI, and 2.59 to 5.34 for LAI at R0-R3.

## 7. PRACTICAL UTILIZATION OF RESULTS

1. Breeding hybrids with enhanced tolerances to fungal infection and higher yield may help manage mycotoxin contamination with lower moderate nitrogen rates of 90 kg ha<sup>-1</sup>.
2. The selection of hybrids demonstrates considerable potential in bridging the yield gap throughout different crop cultivation years, particularly under diverse weather conditions. This is further enhanced by implementing moderate nitrogen application rates of 90 kg N/ha.
3. Visual assessment of *Fusarium graminearum* infection can be used to predict the resultant mycotoxin while being controversial for *Aspergillus flavus* mycotoxins, particularly AFB1.
4. Considering overall measured mycotoxin under natural conditions (un-inoculated treatments) was lower than the European MTL for unprocessed maize for DON <1750 µg kg<sup>-1</sup>, FB1+2, ≤ 4000 µg kg<sup>-1</sup> except for AFB1 >5 µg kg<sup>-1</sup>. The reduction of mycotoxin contamination by integrating hybrid selection and moderate N fertilization demonstrated by hybrid DKC4590 in inoculated treatment offers a potential for reducing mycotoxin under natural conditions, provided that commercial hybrid information on fungal tolerance levels is disclosed.
5. Using non-destructive precision agricultural tools such as chlorophyll meter, remote sensing technologies, and NDVI profiling can be valuable for farmers in assessing maize's health and nutrient requirement, particularly during VT, and allow for timely interventions such as nutrient supplementation.
6. With crop year variability, moderate N rates performed better, surpassing the higher N rate of N150 during the dry year 2022. In contrast, there were no differences between N90 and N150 between 2023 and 2024, with moderately higher rainfall. This suggests that, in rich chernozemic soils, a moderate N rate yields better performance in restricted and adequate water conditions.

## 8. SUMMARY

This study established field experiments to evaluate the effects of applying agrotechnical practices deploying hybrid varieties selection and nitrogen fertilization on yield, *Fusarium* and *Aspergillus* fungal diseases development and examining the resulting mycotoxins contaminations of maize grown in years with varying weather conditions between 2022-2024 cropping seasons. *Aspergillus flavus*, *Fusarium graminearum*, and *Fusarium verticillioides* were the specific fungal species used to evaluate the respective mycotoxins AFB1, DON, and FBs. The implications of relative chlorophyll content using SPAD reading and canopy reflectances of LAI and NDVI measured non destructively were assessed concerning the examined factors inferring for yield prediction.

Established in rich chernozemic soil at the Látókép long-term experiment site of the University of Debrecen, the study involved nitrogen treatment in three (3) regimes (N0, N90, and N150 kgNha<sup>-1</sup>) and three (3) selected maize hybrids. The maize hybrids obtained from commercial companies in Hungary include (DKC4590- FAO360, GKT376-FAO360, and P9610- FAO340), characterized by enhanced tolerance, low tolerances, and unknown sensitivity to fungal disease and mycotoxins contamination, respectively. The latter is additionally recorded as a high-yielding variety.

The results provide a detailed analysis of the effects of hybrid varieties and nitrogen fertilization on maize production and their subsequent impact on the colonization and progression of *Fusarium* and *Aspergillus* fungal ear rot diseases and mycotoxin contamination. The analysis delves into how variability in weather conditions during the growing seasons shifts the situation. Moreover, the understanding and implications of physiological parameters such as relative chlorophyll content and canopy reflectance indices are examined to yield outcomes, fungal disease occurrence, and mycotoxin contamination levels.

The variability in weather conditions during the growing season significantly influenced yield, grain quality, and hybrid performance in mycotoxin contamination. Provided rich soils, the years characterized by low precipitation levels indicated the lowest yield than years with more rainfall, an obvious no surprise. However, the sensitivity of selected maize hybrids in bridging the yield gap was profoundly demonstrated in the year with low

precipitation. The hybrid DKC4590 surpassed other hybrids across all nitrogen regimes (N0, N90, and N150), showing the highest performance at the moderate N90 level. Stability with the hybrid DKC4590 remained similar and consistent in the succeeding years, with the highest performance in 2024 at the N90 level. With adequate water, hybrid GKT376 least performed at N0 in 2023. Its performance at N150 was also medium, with no variation with DKC4590. At the same time, P9610 yielded the highest, although 2024 recorded the highest yield of GKT376 at N150, explaining GKT376's lack of stability in varying weather and fertilization conditions. Therefore, this study suggests hybrid selection with stability across varying weather conditions and nitrogen fertilization, advocating hybrid DKC4590 and a moderate N rate of N90.

Further, the variability in weather conditions resulted in a shift in mycotoxin contamination. The year characterized by relatively less rainfall and increased temperature in 2022 recorded elevated AFB1 at 40.28 ppb, while DON dominated in 2023 at 56.59 ppm, a year characterized by the highest precipitation and lowest temperature, also recording negligible AFB1. The year 2024 is characterized by moderate rainfall compared to other years, and the highest temperature recorded higher AFB1 (36.35) ppb and lowest DON. However, no variation was recorded for FBs contamination between the years, recording an average of 2.15, 2.71, and 2.73 ppm in 2022, 2023, and 2024, respectively.

In studying the effects of hybrid selection and nitrogen fertilization on fungal disease development and mycotoxin contamination, the study indicated increasing AFB1 and FBs with N rate in 2022, while no impacts on DON, similar to 2024. However, in 2023, DON was recorded to decrease with the N rate. Integrating the N rates with hybrid selection indicated the defined performance of hybrids over nitrogen fertilization. Demonstrating stability across N levels in the varying weather conditions across the year, The hybrids DKC4590 showed a reduction in mycotoxin at a moderate N rate of N90.

The hybrid DKC4590 was found to perform better with consistency in yield and mycotoxin contamination across the years. This observation highlights the importance of selecting hybrids with documented agronomic traits and various stress tolerances, particularly the development of fungal diseases and mycotoxin contamination. It is particularly relevant when fluctuations in weather conditions can significantly influence outcomes. Therefore,

under natural conditions, the current study advocates opting for such hybrids under moderate nitrogen fertilization as a possible strategy to reduce mycotoxin contamination while simultaneously addressing yield gaps.

In yield prediction, using relative chlorophyll (SPAD) reading and other canopy reflectance indices of LAI and NDVI measured across various growth stages gives an indicative yield of hybrids under nitrogen treatment. The measured indices indicated a positive correlation with yield and were higher, particularly during VT, followed by R0-R3. The relationship suggests that these indices, measured non destructively, can be used by producers to predict yield in smart agricultural technologies. However, the relationship between fungal year rots and mycotoxin contamination was not indicative. It did not show defined trends of the relationships between varying growth stages indices on fungal diseases and mycotoxin contaminations; hence, further comprehension is needed to give inferences.

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## 10 PUBLICATIONS



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Subject: PhD Publication List

Candidate: Muhoja Sylvester Nyandi  
Doctoral School: Kálmán Kerpely Doctoral School  
MTMT ID: 10086952

### List of publications related to the dissertation

#### Hungarian scientific articles in Hungarian journals (1)

1. Pepó, P., **Nyandi, M. S.**: A mikotoxinok Magyarország és Tanzánia növénytermesztésében. 77 (17), 28-29, 2022. ISSN: 0025-018X.

#### Foreign language scientific articles in Hungarian journals (2)

2. **Nyandi, M. S.**, Pepó, P.: Effects of N fertilization and hybrid genotype on fusarium and aspergillus mycotoxin contamination in maize kernels. *Cereal Res. Commun. Epub ahead of print*, 1-9, 2024. ISSN: 0133-3720.  
DOI: <http://dx.doi.org/10.1007/s42976-024-00599-z>  
IF: 1.6 (2023)
3. **Nyandi, M. S.**, Pepó, P.: The roles of mycotoxins in cereal crops production: A comparative study of Hungary and Tanzania. *Agrártud. közl.* 2022 (1), 151-159, 2022. ISSN: 1587-1282.  
DOI: <http://dx.doi.org/10.34101/actaagrar/1/10833>

#### Foreign language scientific articles in international journals (2)

4. **Nyandi, M. S.**, Pepó, P.: Aspergillus and Fusarium Mycotoxin Contamination in Maize (Zea mays L.): The Interplay of Nitrogen Fertilization and Hybrids Selection. *Toxins*. 16 (7), 1-14, 2024. EISSN: 2072-6651.  
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In: 11th International Symposium on Recent Advances in Food Analysis : Book of abstracts. Eds.: Jana Pulkrabová, Monika Tomaniová; Stefan van Leeuwen; Michele Suman; Michel Nielen, Jana Hajšlová, The University of Chemistry and Technology, Prague, 451, 2024. ISBN: 9788075922687
7. **Nyandi, M. S.**, Pepó, P.: Influence of nitrogen fertilization and hybrids on chlorophyll content and consequent maize grain yield.  
In: 21st Wellmann International Scientific Conference Book of abstracts / felelős kiadó Edit Mikó, szerk. Ingrid Melinda Gyalai, Szilárd Czóbel, University of Szeged Faculty of Agriculture, Hódmezővásárhely, 20, 2024. ISBN: 9789633069806
8. **Nyandi, M. S.**, Pepó, P.: Responses of selected maize genotypes (*Zea mays* L.) on nitrogen fertilization: grain yield and quality attributes.  
In: 20th Wellmann International Scientific Conference : Book of Abstracts. Eds.: Ingrid Gyalai; Szilárd Czóbel, University of Szeged Faculty of Agriculture, Hódmezővásárhely, 38, 2023. ISBN: 9789633069240
9. **Nyandi, M. S.**: The role of nitrogen fertilization on fungal colonization and severity of ear rot on selected maize genotypes (*Zea mays* L.): inoculation of *Aspergillus flavus* and *Fusarium verticillioides*.  
In: Scientific Conference of PhD Students of FAFR, FBFS and FHLE SUA in Nitra with international participation - Proceedings of abstracts on occasion of the Science and Technology Week in the Slovak Republic. Ed.: Monika Tóthová, Judita Lidíková, Kristína Candráková, Dominik Holly, Slovak University of Agriculture in Nitra, Slovakia, Nitra, 51, 2023. ISBN: 9788055226606

**List of other publications**

Hungarian scientific articles in Hungarian journals (2)

10. Pepó, P., **Nyandi, M. S.**: Néhány agrotechnikai tényező hatása a kukorica (*Zea mays* L.) szemtermésének kémiai összetételére.  
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DOI: <http://dx.doi.org/10.3389/fpls.2023.1232675>  
IF: 4.1

**Total IF of journals (all publications): 12,8**

**Total IF of journals (publications related to the dissertation): 5,5**

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

03 April, 2025



## 11. DECLARATIONS

### DECLARATION

This dissertation enables to obtain the doctoral (Ph.D.) degree of the University of Debrecen prepared in the framework of Kerpely Kálman Doctoral School of the University of Debrecen.

Debrecen, 20.....

.....

Signature of the candidate

### DECLARATION

I certify that ..... doctoral candidate 20 .....-20 ..... The above-mentioned Doctoral School carried out its work under my direction. The independent creative activity of the candidate is decisive for the results included in the dissertation, and the dissertation is the candidate's independent work. I suggest/recommend the acceptance of the dissertation.

Debrecen, 20 .....

.....

Signature of the supervisor (s)

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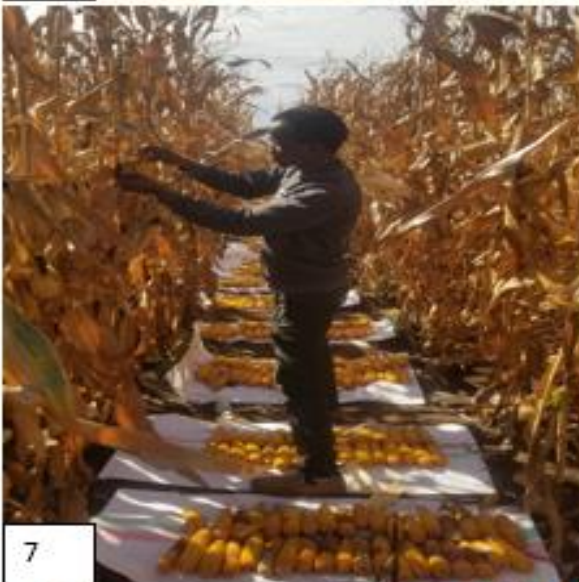
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## ACKNOWLEDGEMENT

Above all, I am thankful to the almighty GOD for granting me good health throughout the study during the stay in Debrecen, Hungary.

I would like to express my sincere gratitude and appreciation to my supervisor Prof. Dr. PÉTER PEPÓ (DSc.) for his unwavering and constructive guidance, encouragement and insightful support rendered throughout the study process from proposal development, experiment establishment, data collection and preparation of this dissertation.

A debt of gratitude goes to all staff of the institute of plant production for their invaluable support and collaboration throughout my academic journey, their invaluable support, expertise and generosity in knowledge sharing greatly enriched this journey with a vital role to successful completion. I am grateful and thankful to my thesis opponents Prof. Dr. CSAJBÓK JÓZSEF and Dr. FUTÓ ZOLTAN (Ass. Professor) for being resourceful and constructive criticism and feedback, which comprehensively enhanced the overall quality of the dissertation.

I am grateful to my family, my late parents LETICIA RAPHAEL and SYLIVESTER NYANDI who built the foundation in my academic journey. I am profoundly grateful to my wife Mrs. HYASINTA CONSTANTINE MWANGA for her unwavering support, patience and understanding throughout this journey. Her belief in me and encouragement strengthened me for making this accomplishment.

To my friends and colleagues, ANTENEH AGEZEW MELASH, BEKIR BYTYQI, EBENEZER AYEWE APPIAH, AKASAIRI OCWA and everyone else I had the opportunity to interact with and who have contributed to my pleasant stay and memorable experiences in Hungary, thank you very much. I would like to express my sincere gratitude to the Látókép Research site Staff of the university of Debrecen for their technical guidance and support throughout the experiment