Thesis of a Doctoral (PhD) Dissertation

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

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Debrecen
2025

1. INTRODUCTION

1.1 Background and Rationale

Maize (Zea mays L.) has become crucial in global agri-food systems 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. 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 . In SSA, South America, and parts of Asia, maize is vital for human consumption, providing about 20% of food calories. 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. Extreme weather events like heat waves, droughts, and heavy rainfall are becoming more common. 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. 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 & 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, directly influencing yield and grain quality. 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 be contaminated with mycotoxins at different levels, making mycotoxin a worldwide food safety and public health problem. Research and outreach efforts by public-private entities and NGOs are continuously being carried out to address all aspects of the prevention and mitigation of mycotoxins. The efforts include several global comprehensive projects funded by the European Commission, including MycoGlobe(2004-2008), MycRed(2012-2016), MyToolBox (2016-2020), MycoKey(2016-2020) (www.mycokey.eu). The projects aim at developing and testing smart integrated sustainable solutions and innovative tools to reduce the significant mycotoxins in economically important food and feed chains, including explicit development and implementation of rapid, reliable, and validated tools for detection of toxigenic fungus. Maize is often affected by three main toxin-producing fungi, namely, *Fusarium graminearum,* which produces deoxynivalenol (DON) and zearalenone (ZEA), *Fusarium verticillioides* producing fumonisins toxins and *Aspergillus flavus* which produces aflatoxins. 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.

The prevention of mycotoxins hinges on managing mold-producing fungi, which necessitates implementing strategies both before and after the harvest. 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 and the tolerance levels of the hybrids Additionally, the contamination of maize with mycotoxins is significantly affected by agronomic practices employed in the cultivation process.

Finding resistant maize genotypes has been the primary tactic for combating the issue. 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. Screening of tolerance to some commercial hybrids has been reported, 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. 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. Therefore, balancing nitrogen levels in soil and avoiding deficiencies or excesses is crucial for managing mycotoxin risks, as nutrient imbalances can aggravate susceptibility to pests and diseases.

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. 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

This 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 to assess 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, fungal ear rot severity, 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, fungal diseases development, and mycotoxin contamination of maize hybrids under nitrogen treatments

2. MATERIALS AND METHODS

2.1 Experimental site

The three-year experiment was set up in 2022, 2023, and 2024 spring at the Látókép long-term experiment site of the University of Debrecen, located 47°33'42" N; 21° 27'02" E, about 15 km from Debrecen. The long-term experiment station was set up in 1983, initially to conduct experiments on fertilization and crop rotation. The soil conditions at the site are characterized by homogenous calciferous chernozem developed on the Hajdúság loess ridge. The upper layer exhibits an average humus content of 2.7% to 2.8%, with a thickness of approximately 0.8 meters. The acidity levels in the upper soil strata are near neutral, indicated by a pH_{KCl} range of 6.46 to 6.6. The phosphorus availability in this calcareous soil can be classified as average, with AL-soluble P_2O_5 measured at 133 mg kg^{-1}. The potassium supply falls into the average-good category, with AL-soluble K_2O recorded at 240 $mgkg^{-1}$. The soil's plasticity index (KA) also varies between 43 and 47.6. Further, wheat served as the forecrop at the experimental site.

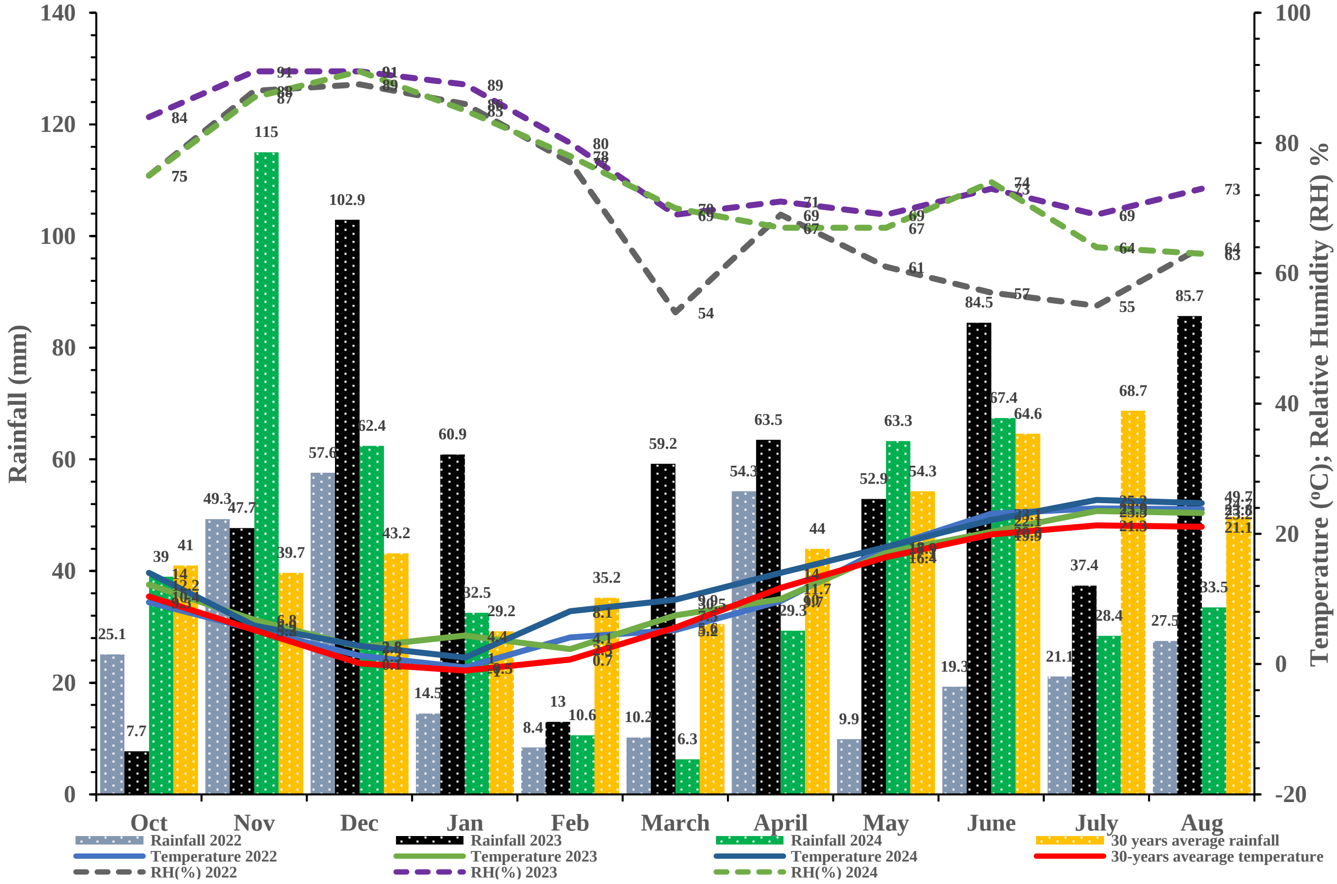


Figure 1: Meteorological data of the Látókép research station during the experiment years and the average for the past 30 years (Debrecen: 2022, 2023 & 2024).

In all growing seasons, the soil moisture content present at the sowing was suitable for seed germination, and 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. One was performed in May and another in early July. These critical interventions occurred during key physiological stages, such as vegetative, flowering (Tasselling and silking), and grain filling. Figure (2) illustrates the general climatic and weather conditions observed during the experimental crop years alongside the average patterns recorded over the preceding 30 years.

2.2 Experimental design and treatments

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 three maize hybrids, DKC4590 (FAO 360), GKT376 (FAO 360), and P9610 (FAO 340) 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 nitrogen rates (0, 90, and 150 kgNha^{-1}, and the sub-subplot treatment was the fungal ear inoculation. The subplot size was 5 m × 3.04 m (15 m^2) with an interrow spacing of 76 cm, making four rows plot^{-1} consisting of approximately 25 plants each and a distance of 1 m between blocks. Three rows were exposed to artificial inoculation of fungal isolates; according to international guidelines, one inoculum was employed for a single pathogen. One with a strain of *F. graminearum* that produces deoxynivalenol (DON), one with a strain of *F. verticillioides* that produces fumonisins (FBs)$_{\textbf{total}}$, and the third raw sample was inoculated with a strain of *A. flavus* that produces aflatoxins (AFBs), AFB1 measured. The untreated check was the fourth raw.

2.3 Isolates and Inoculation

All strains of *F. graminearum, F. verticillioides,* and *A. flavus* used in this study were sourced from naturally infected grain in Hungary and are part of the microorganism collection at Cereal Research Nonprofit Ltd (Gabonakutató Nonprofit Kft) in Szeged, Hungary. The inocula was prepared following the guidelines established by Szabó et al. (2018). Subsequently, inoculation was carried out using the toothpick method developed by Young (1943), as modified by Mesterházy (2008), to assess kernel resistance (Figure 2). In all experimental years, inoculation was performed 6 days after 50% mid-silking by inserting infested toothpicks into the middle of the upper ear in a hole made by an awl 15-mm-long and 1.5-mm wide and left until the harvesting period.

Figure 2: Illustrates the toothpick method inoculation process, depicting the sequence of steps: (1) Inoculum toothpicks, (2) Creating an aperture, (3) Punctured ear, (4) Inoculum toothpick inserted

2.4 Data collection procedures

2.4.1 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 inoculated cobs per plot for each fungal isolate. Upon reaching maturity, with a permissible harvest moisture content near 20%, the cobs were harvested manually and dehusked to assess the extent of ear and kernel rot. The evaluation of fungus-induced ear rot severity was quantified using a percentage scale (Mesterházy et al., 2022). The incidence is expressed as the proportion of ear coverage on an average regular ear, which contains 700-800 grains. Therefore, a 1% infection is determined when the ear presents 7-8 kernels displaying visible signs of infection, whereas a 0.15% infection is identified when merely one kernel exhibits visible infection. The severity assessment incorporated two data categories: one indicating direct infection from the toothpick method, and the other recorded naturally occurring infection independent of this method.

Figure 3: Ears inoculated with fungal isolates of FG, AF, and FV vs untreated controls

2.4.2 Sample preparations and measurement of mycotoxins

In mycotoxin analysis, five ears exhibiting an average 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 contamination was assessed through an enzyme-linked immunosorbent assay (ELISA) utilizing ELISA kits produced by Romer Labs, Tulln, Austria. The quantification of total fumonisin (FBs), deoxynivalenol (DON), and aflatoxin B1 (AFB1) was performed accordingly by;

- *i) AgraQuant Fumonisin 0.25/5.0 ELISA kit,*
- *ii) AgraQuant Deoxynivalenol 0.25/5.0 ELISA kit and,*
- *iii) AgraQuant Aflatoxin B1 2/50*

Using a direct competitive assay, the samples were evaluated at a wavelength of 450 nm using a microtiter plate reader: Thermo Scientific, Waltham, Massachusetts, USA. Each measurement was conducted 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).

2.4.3 Measurement of Physiological and Quality Parameters

2.4.3.1 Relative Chlorophyll Content (SPAD index)

The leaves' relative chlorophyll concentration and nitrogen status were measured with a handheld chlorophyll meter (Minolta SPAD-502). The ten youngest fully expanded leaves were randomly chosen at each test unit for measurement from the two central rows of each plot. The non-destructive measurement began 50 days after sowing and continued throughout the growth season. The device evaluates leaf transmittance at wavelengths of red light (650 nm) and NIR (940 nm). The SPAD meter calculates unit values for leaf chlorophyll concentration as described by Wood et al. (1993) in the equation (i)**;**

$$\boldsymbol{SPAD\ Index = A \times \left[\log\left(\frac{Ior}{Ir}\right) - \log\left(\frac{Iof}{If}\right) + B\right]} \text{...}\mathbf{(i)}$$

Where:
A; B = constants;
Ior = current from red detectors with a sample in place;
Ir = current from red infrared detectors with sample in place;
Iof = currents from red detectors with no sample and,
If = currents from infrared detectors with no sample

2.4.3.2 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^{-2} s^{-1}) and LAI units (m^2 m^{-2}).

2.4.3.3 The Normalized Difference Vegetation Index (NDVI)

NDVI was assessed during various stages of development using the "Trimble Greenseeker" NDVI meter. 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). Readings were taken for the plot with the equipment positioned 1.0 m above the maize canopy.

NDVI = (NIR - RED)/ (NIR+ RED)..(ii)

2.4.3.4 Harvesting and Grain Yield

The grain yield in a 7.6 m^2 plot was measured and converted into kg ha^{-1} by a two-row Sampo Rosenlew SR 2010 plot combine harvester (Figure 5) using a Coleman weighing system. The grain yield was adjusted to 14% moisture content following Badu-Apraku et al. (2012), as shown in equation (iii),

Grain yield (kg ha^{-1}) = Grain yield x(100 - % AMC)/ (100 - % SMC) × 100.......................(iii)

Where:

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

SMC = Standard Moisture Content

2.4.3.5 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.

2.5 Statistical Data Analysis

The impact of nitrogen rates 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 Genistat 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 to investigate the intricate interrelationships among various variables.

3. RESULTS

3.1 Yearly variation in agrometeorological conditions influences maize yield and mycotoxin contamination

The yearly agrometeorological conditions during the maize growing seasons of 2022, 2023, and 2024 are presented (figure 4). 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 moisture content at the sowing was suitable for germination, 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.

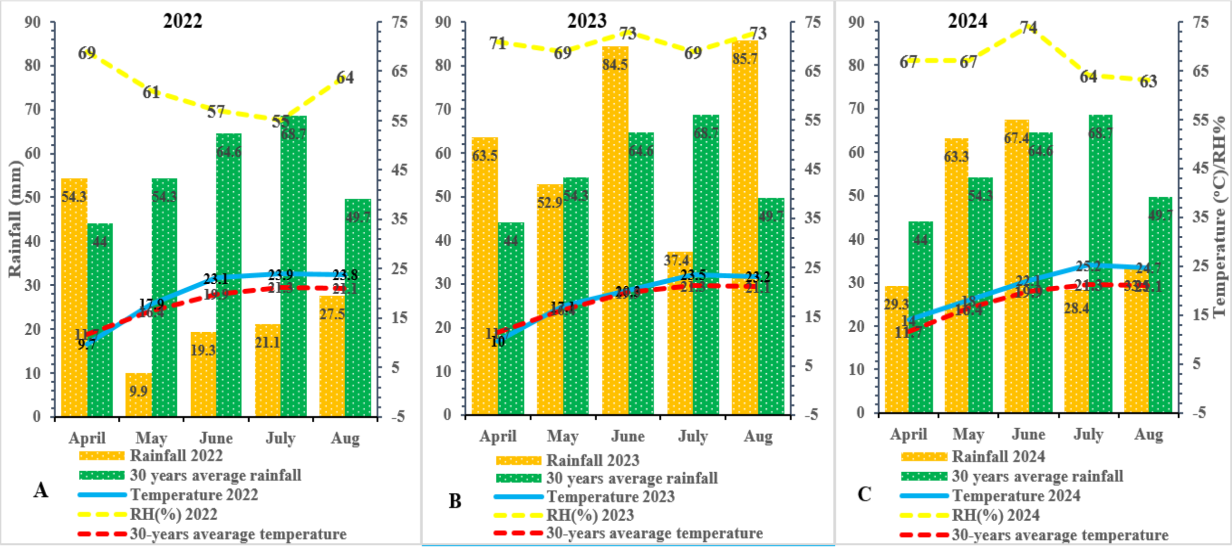


Figure 4: Yearly agrometeorological conditions during the growing seasons at the Latókép experiment site of the University of Debrecen (2022-2024): A&D, B&E, C&F Rainfall and Temperature: RH and Temperature for 2022, 2023, and 2024 respectively).

All the growing seasons, 2022, 2023, and 2024, experienced a temperature above the average of the past 30 years (table 1), 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 1: Deviation of weather conditions in growing seasons (Debrecen, 2022-2024)

Year	Total year rainfall	Rainfall in growing season (mm)	Deviation from the 30 yrs. average	Temperature (ºC)	Deviation from the 30 yrs. 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		

3.1.1 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 2). 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 2: Crop year weather variability influences grain yield and quality

Source of variation	Yield (kg ha^{-1})	Starch (%)	Protein (%)
2022	7638	71.50	10.60
2023	12793	64.34	7.13
2024	20755	64.81	7.50
LSD$_{(0.05)}$	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 2). 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 5). Bojtor et al. (2021) stressed that yield is affected by interactions between crop year variations, nitrogen fertilization practices, and hybrid selection strategies.

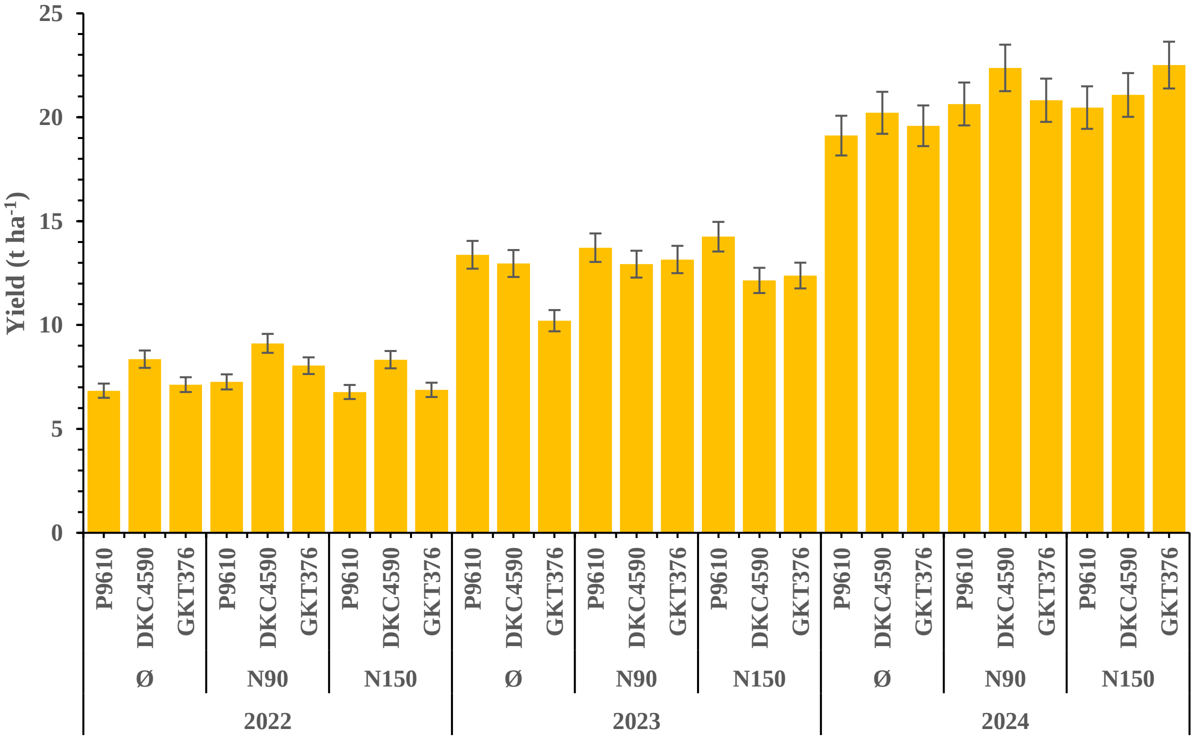


Figure 5: Interactive effects of nitrogen fertilization and selected hybrids on yield across the experiment years (Debrecen, 2022-2024)

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 5). 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).

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.

3.1.2 Yearly variation in agrometeorological conditions influences fungal disease development and mycotoxin contamination

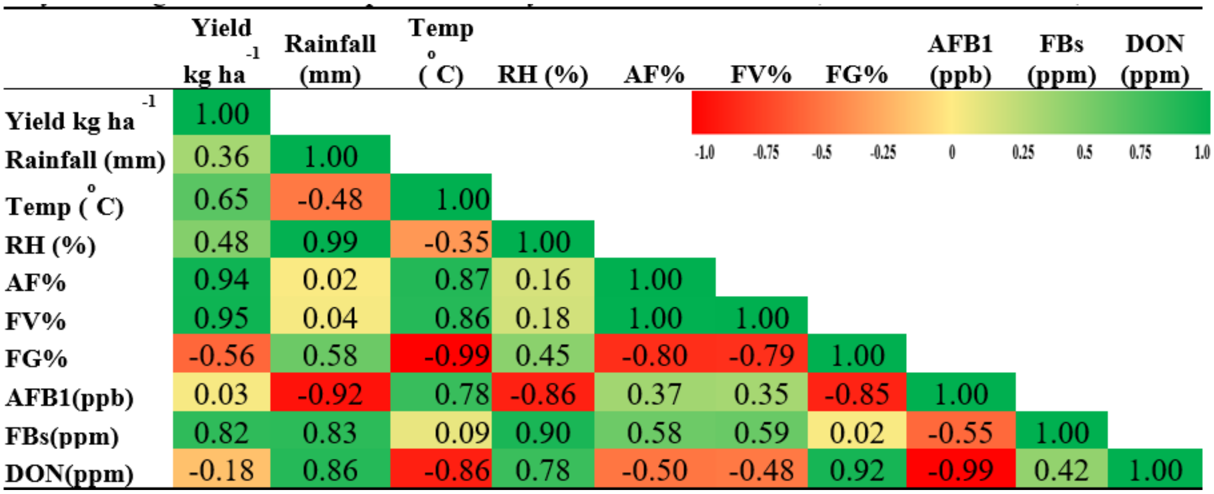
The effect of annual agrometeorological conditions on the development of fungal diseases and mycotoxin contamination is illustrated in Table 3. Additionally, Table 4 provides a detailed analysis of the complex interactions, as evidenced by Pearson's correlations, between yearly variations and weather elements concerning ear rot infection and mycotoxin contamination.

Table 3: 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
$LSD_{(0.05)}$	0.06	0.08	2.85	20.73	0.75	14.23

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.

Table 4: Coefficient values of Pearson correlation showing a relationship of yearly agrometeorological conditions on yield, fungal disease development, and mycotoxin contamination (Debrecen, 2022-2024)



	Yield kg ha^{-1}	**Rainfall (mm)**	**Temp (°C)**	**RH (%)**	**AF%**	**FV%**	**FG%**	**AFB1 (ppb)**	**FBs (ppm)**	**DON (ppm)**
Yield kg ha^{-1}	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

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 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. This confirms the previous study showing that reduced rainfall and warmer conditions facilitate the colonization of *A. flavus* and respective mycotoxin contamination, corroborating Giorni et al. (2016). Further, Kos et al. (2024) indicated that aflatoxins were present in 94% of the maize samples collected in 2012, the year noted extreme drought in Serbia exhibiting concentration levels ranging from 0.6 to 205 µg/kg.

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 3). This indicates that the variability in weather conditions during this study did not influence the production of fumonisin toxins.

3.2 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 5). Relative chlorophyll content differences existed among maize hybrids at different growth stages, regardless of specific nitrogen rate and crop year. 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).

Table 5: 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**	**Protein**	**Starch**
		V6	**VT**	**R0-R3**	**R6**	**AVG**	**(Kg/ha)**	**(%)**	**(%)**
Nitrogen fertilization rates									
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
	LSD$_{(0.05)}$	**2.63**	**1.784**	**1.868**	**3.555**	**1.256**	**1374.6**	**0.5828**	**1.822**
Maize Hybrid effects									
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
	LSD$_{(0.05)}$	**2.572**	**1.802**	**1.78**	**2.873**	**0.987**	**1231.9**	**0.6144**	**1.795**

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.

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

The LAI indices across various growth stages were significant (P<0.05) within hybrids and N rates in the years. Similarly, 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 6). The various growth stages of the crops exhibited pronounced impacts on Leaf Area Index (LAI) and Normalized Difference Vegetation Index (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 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 6: 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**			
Nitrogen fertilization rates														
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
	LSD$_{(0.05)}$	**0.280**	**0.262**	**0.379**	**0.389**	**0.172**	**0.057**	**0.029**	**0.020**	**0.057**	**0.026**	**1375**	**0.583**	**1.822**
Maize Hybrid effects														
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
	LSD$_{(0.05)}$	**0.389**	**0.280**	**0.346**	**0.269**	**0.208**	**0.111**	**0.033**	**0.025**	**0.035**	**0.038**	**1232**	**0.614**	**1.795**

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.

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 that prevailed 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.

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.

3.3 N fertilization, hybrids, and inoculation affect ear rot severity and mycotoxin levels.

Table 7 illustrates the effects of nitrogen fertilization and hybrid tolerance 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. 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. Inoculation treatments significantly affected % ear rot severity and produced mycotoxins across all nitrogen rates and hybrid tolerances in all years. Both fungal infection and mycotoxin were heightened in inoculated treatment compared to non-inoculated controls.

Table 7: Effects of nitrogen fertilization, hybrids, and inoculation treatments on ear rot severity and mycotoxin contamination (Debrecen, 2022-2024)

Factors for variation		**The severity of ear rot**			**Mycotoxins Amounts**		
		AF%	**FV%**	**FG%**	**AFB1(ppb)**	**FBs(ppm)**	**DON(ppm)**
N application doses							
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
	LSD$_{(0.05)}$	**0.11**	**0.13**	**5.59**	**33.49**	**1.26**	**26.96**
Maize hybrid effects							
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
	LSD$_{(0.05)}$	**0.11**	**0.12**	**5.40**	**35.08**	**1.16**	**32.27**
Inoculation effects							
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
	LSD$_{(0.05)}$	**0.08**	**0.11**	**4.45**	**27.49**	**1.03**	**25.00**

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

3.4 Interactions between Nitrogen Fertilization and Hybrids and Mycotoxin Contamination

The interactive effect of nitrogen fertilizer application rate and hybrid genotypes on the amount of mycotoxin contamination evaluated is shown in Table 8. The interaction of selected hybrids and nitrogen treatments on *A. flavus* mycotoxin contamination demonstrated a significant ($p < 0.05$) in 2022 across fertilizer rates and hybrids

Table 8: Interactive effects of nitrogen fertilizer application rate and hybrid genotypes on the amount of mycotoxin contamination

	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
	Mean		**8.11**	**3.02**	**62.59**	**3.1**	**1.29**	**20.21**
LSD$_{(0.05)}$			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
	Mean		**113.17**	**4.66**	*****	*****	**1.16**	*****
LSD$_{(0.05)}$			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
	Mean		**3.93**	**4.53**	**56.20**	**1.02**	**1.67**	**20.04**
LSD$_{(0.05)}$			3.20	3.26	24.86	3.20	3.26	24.86

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

The results indicate that 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.

3.5 Principal Component Analysis: General Associations of the Examined Predictors on Yield, Physiological Parameters, Fungal Diseases Development and Mycotoxins 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 6. 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.

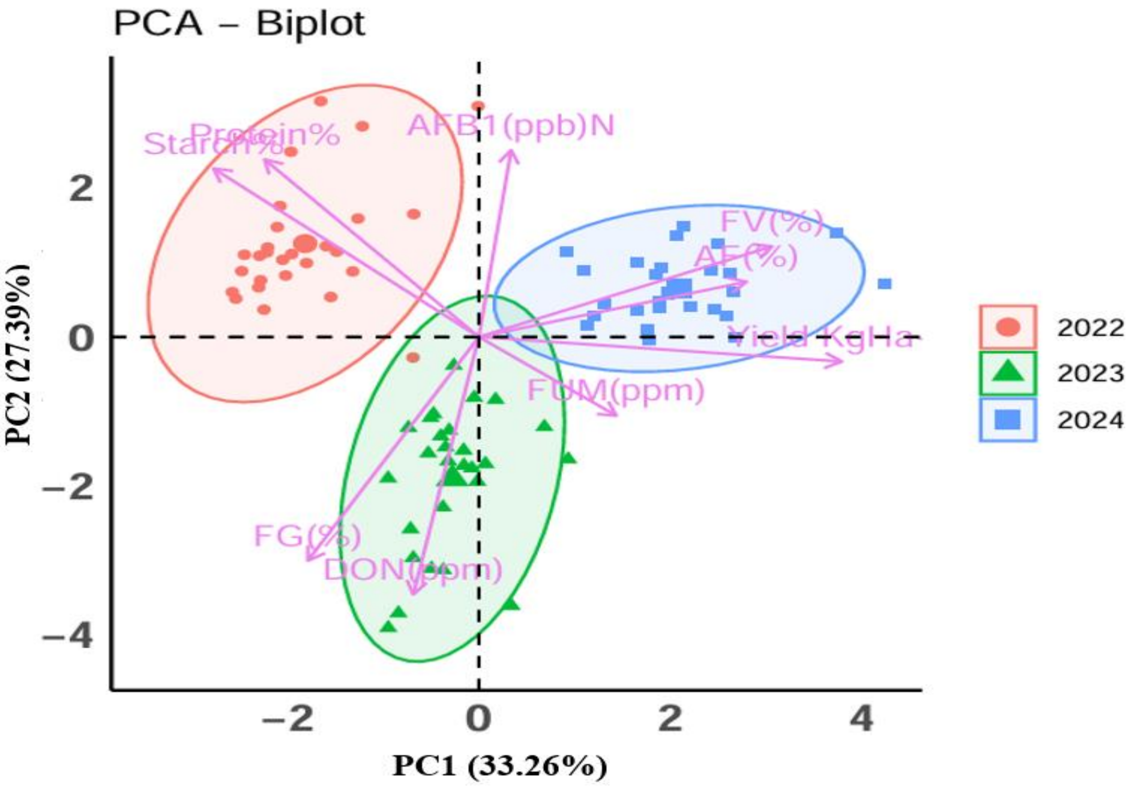


Figure 6: The principal component analysis biplot showing the relationships of crop year on yield, fungal disease development, and mycotoxin contamination. The first principal component explains 33.26% of the variance, while the second principal component contributes 27.39%, making a total variability of 60.65

The 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.

4. 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^{-1} vs. 13.8 and 14 tonsha^{-1} for P9610, and GKT376 at N90 while recording 13.8 tonsha^{-1} vs. 13.86 and 13.9 tonsha^{-1} 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.

5. PRACTICAL UTILIZATION OF RESULTS

1. Breeding for hybrids with enhanced tolerances to fungal infection and higher yield may help manage mycotoxin contamination with lower moderate nitrogen rates of 90 kg ha^{-1}.

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 $\mu g\ kg^{-1}$, FB1+2, $\leq 4000\ \mu g\ kg^{-1}$ except for AFB1 $>5\ \mu g\ kg^{-1}$. 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.

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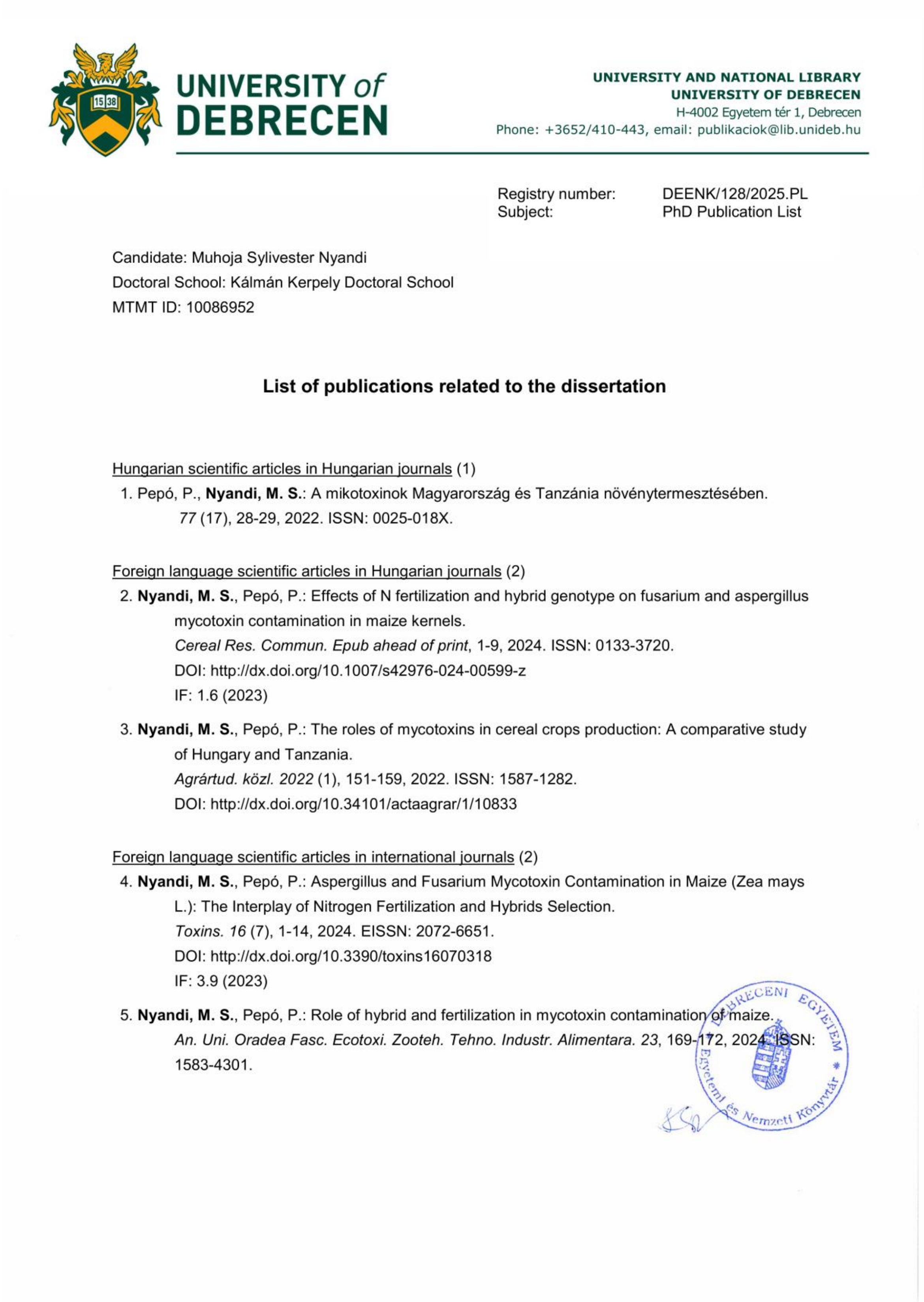
7. PUBLICATIONS



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Registry number: DEENK/128/2025.PL
Subject: PhD Publication List

Candidate: Muhoja Sylivester 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
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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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5. **Nyandi, M. S.**, Pepó, P.: Role of hybrid and fertilization in mycotoxin contamination of maize.
An. Uni. Oradea Fasc. Ecotoxi. Zooteh. Tehno. Industr. Alimentara. 23, 169-172, 2024. ISSN: 1583-4301.

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6. **Nyandi, M. S.**, Pepó, P.: Assessing the implication of SPAD and NDVI Values in Nitrogen Application and grain quality of selected maize hybrids.
   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 vertcilioides.
   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 Lidiková, 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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11. Pepó, P., **Nyandi, M. S.**: Lehet-e tanulni a fejlődő országok mezőgazdaságából? Összehasonlító elemzés Magyarország és Tanzánia növénytermesztéséről.
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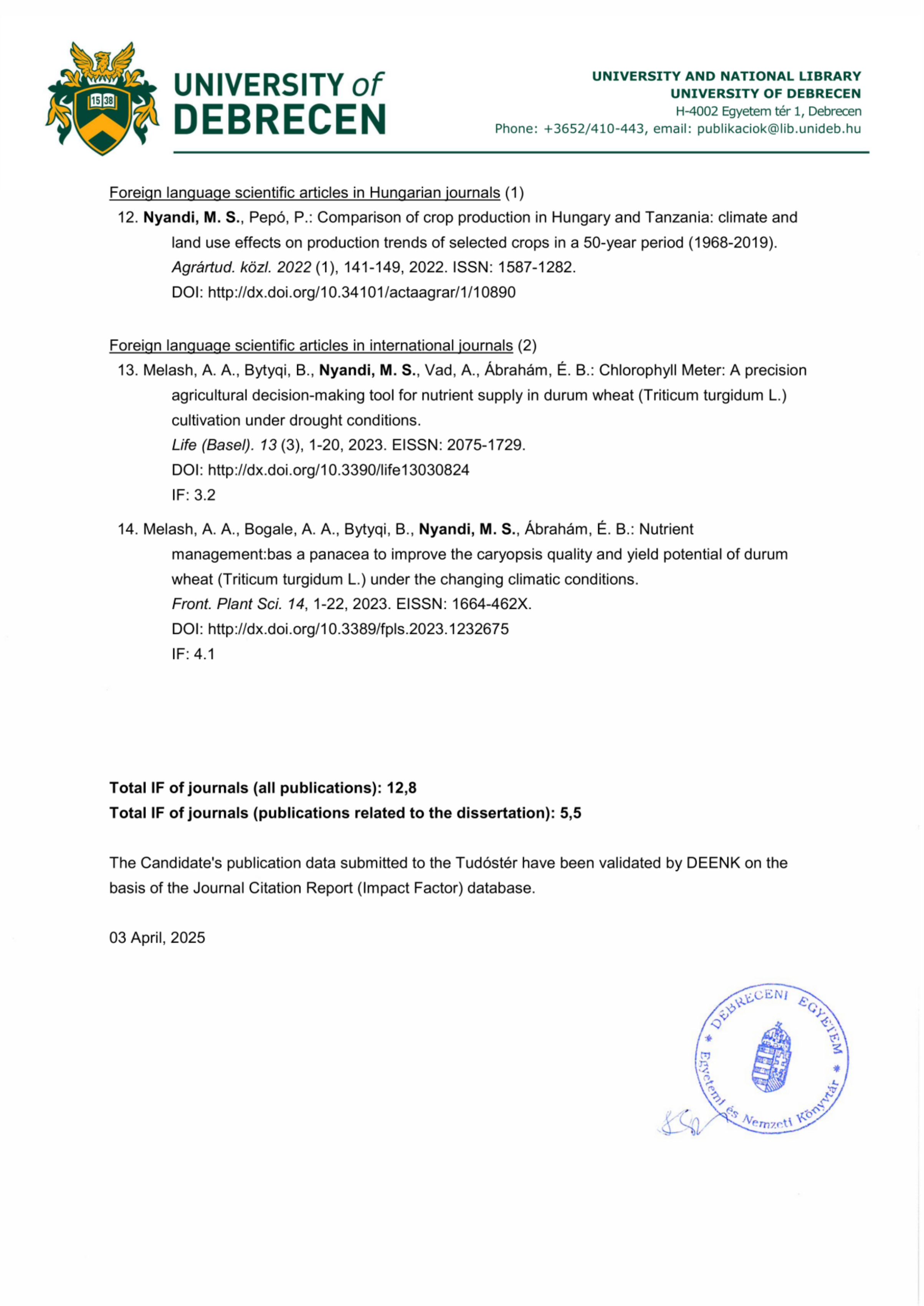

Foreign language scientific articles in Hungarian journals (1)

12. **Nyandi, M. S.**, Pepó, P.: Comparison of crop production in Hungary and Tanzania: climate and land use effects on production trends of selected crops in a 50-year period (1968-2019).
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    DOI: http://dx.doi.org/10.34101/actaagrar/1/10890

Foreign language scientific articles in international journals (2)

13. Melash, A. A., Bytyqi, B., **Nyandi, M. S.**, Vad, A., Ábrahám, É. B.: Chlorophyll Meter: A precision agricultural decision-making tool for nutrient supply in durum wheat (Triticum turgidum L.) cultivation under drought conditions.
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    Front. Plant Sci. 14, 1-22, 2023. EISSN: 1664-462X.
    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

ACKNOWLEDGEMENT

Above all, I am thankful to the almight 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 AYEW 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