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Harnessing chlorophyll and canopy reflectance indices relationship for grain yield, protein and starch content in maize cultivars under different nitrogen treatments

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Abstract: Crop production faces increased climate change and land degradation stresses, compromising global food security with the growing population. Maize (*Zea mays* L.) is a versatile crop used for food, feed, and raw materials, contributing significantly to global food systems. Abiotic stresses like drought and soil fertility limit its production. Fertilisation is an amelioration technique that optimises maize growth and yield by maintaining optimum nutrition and leveraging nutrient deficiency conditions. Precision agricultural tools like chlorophyll meters are essential for non-destructive chlorophyll assessment and nitrogen status. An experiment conducted at the University of Debrecen evaluated the impact of nitrogen (N) fertilisation (0, 90, and 150 kg/ha) and three maize cultivars (P9610-FAO 340, DKC4590-FAO360, and GKT376-FAO360) on physiological parameters, namely: relative chlorophyll content (SPAD), normalised differences vegetation index (NDVI) and grain quality. Results showed that SPAD and NDVI positively correlated ($P < 0.05$) with grain quality and yield. Nitrogen application significantly influenced SPAD. Maize cultivars and N rates with higher chlorophyll content had maximum yield. Cultivar responses to nitrogen rates significantly ($P < 0.05$) varied by crop year. Higher SPAD and NDVI values were associated with higher protein content. Therefore, SPAD and NDVI values could be used to analyse the nutrient requirements of maize under field conditions to estimate grain yield.

Keywords: macronutrient; spectrometry; phenotyping; remotesensing; bioindicator; hybrid selection

Global crop production is currently greatly affected by climate variability and land degradation, compromising global food security. Maize is a crucial global crop used for food, feed, and industrial purposes. Abiotic stresses such as drought and soil fertility hamper maize productivity. Fertilisation is a method to optimise nutrient availability and address deficiencies for optimal crop growth. Nitrogen (N) is essential for grain crop growth and productivity and is a major limiting factor in their production. Nitrogen fertiliser influences the growth of maize plants by affecting the development and maintenance of leaf area and their photosynthetic capacity (Geith et al. 2022). This, in turn, directly affects the yield and quality of the grain produced by crop plants (Gao et al. 2020). The grain-yielding capability of maize depends on the crop's capacity to assimilate CO_2 (Correia et al. 2021). The nitrogen content

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of a leaf often correlates with its ability to photosynthesise. This is because most of the nitrogen in a leaf is found in chlorophyll molecules, resulting in a strong connection between leaf chlorophyll content and nitrogen levels (Luo et al. 2021).

Precision agriculture technologies, such as canopy sensing and variable rate application, offer a viable strategy for improving N management in crop production (Shishodia et al. 2020). Canopy sensing offers real-time insights into the crop in the field and can adjust N input based on the actual crop requirements (Berger et al. 2020). The normalised difference vegetation index (NDVI) and chlorophyll meter (SPAD) are commonly used to analyse crop canopy structure and health, as well as leaf chlorophyll content for predicting grain yield and managing nitrogen levels in different crops (Rhezali and Aissaoui 2021, Ali and Salem 2024). A critical assumption in using optical sensing to detect nitrogen stress is that crops with a nitrogen deficiency will absorb less light than crops with sufficient nitrogen, leading to a decrease in light reflected from the nitrogen-deficient crop (Mu and Chen 2021). Therefore, canopy reflectance is a practical, non-destructive tool for quickly assessing the nitrogen levels of plants (Wang et al. 2022). However, more research is needed to explore the combination of plant physiological indices data and grain quality indicators. Additionally, more data is needed to evaluate canopy indices and leaf chlorophyll content as potential indicators for nitrogen levels in maize cultivation when subjected to varying nitrogen levels and different maize cultivars. Therefore, this research aimed to assess how different levels of nitrogen application impact leaf chlorophyll content in maize and evaluate the impact of nitrogen fertiliser application on leaf chlorophyll content, grain yield, and protein content in different cultivars of maize.

MATERIAL AND METHODS

Experimental site. At the University of Debrecen's Látókép long-term study research site located at 47°33'42"N; 21°27'02"E, Debrecen, Hungary, a field experiment was carried out in 2022 and 2023. The soil type is homogeneous calciferous Chernozem formed on the Hajdúság loess ridge. The upper layer has an average humus content of 2.7–2.8% and is around 0.8 m deep. The upper soil layers have nearly neutral acidity (pH_{KCl} = 6.46–6.60). The calcareous soil has an average supply of phosphorus (AL-soluble 58.5 mg P/kg) and an average-good supply of potassium (AL-soluble 199.2 mg K/kg). The soil plasticity index (K_A) range was 43–47.6. Winter wheat was the preceding crop at the experiment site.

Experimental design and treatments. The study was conducted using a tetraplicate split-split plot design. This design was chosen because the focus was on the relationship between nitrogen and grain quality rather than a straightforward comparison of the effects of different levels of N fertiliser on cultivar grain quality. The main plots represented different nitrogen rates (0, 90, and 150 kg/ha), and the subplots were dedicated to 3 commercial maize cultivars (P9610-FAO 340, DKC4590-FAO 360, and GKT376-FAO 360). Each subplot was 5 m × 3.04 m (15.2 m^2) with a row spacing of 76 cm, and each plot contained 4 rows with around 25 plants and 1 m distance between blocks.

Data collection

Relative chlorophyll content (SPAD index). The leaves' chlorophyll concentration and nitrogen status were measured with a handheld chlorophyll meter (Minolta SPAD-502, Tokyo, Japan). The 10 youngest fully expanded leaves were randomly chosen at each test unit for measurement from the 2 central rows of each plot. 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 Eq.:

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

Where: *A*, *B* – constants; Ior – current from red detectors with sample in place; Ir – current from red infrared detectors with sample in place; Iof – currents from red detectors with no sample; If – currents from infrared detectors with no sample.

Leaf area index. The leaf area index (LAI) was assessed using the Delta-T SunScan SS1 COM-R4 portable plant canopy analyser system, equipped with a radio link, developed by Delta-T Devices Ltd. (Cambridge, 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) and LAI units (m^2/m^2).

The normalised difference vegetation index was assessed during various stages of development using

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the "Trimble Greenseeker" NDVI meter (Colorado, USA). 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 the Eq.:

$$NDVI = (NIR - RED)/(NIR + RED)$$

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

All of the physiological parameters SPAD, LAI and NDVI were measured during V6 (6^{th} colar leaves visible stage); VT (tasseling); R0–R3 (Anthesis-Visible silks-Blister stage/Clear liquid in kernel-Milk stage/White milky fluid in kernels), and R6 (maturity stage-black layer at the grain base) stages, respectively. Additionally, the non-destructive measurement began 50 days after sowing (V6) and continued throughout the growth season.

Grain yield. The grain yield in a 7.6 m^2 plot was measured and converted into kg/ha by a Sampo Rosenlew SR 2010 plot combine harvester using a Coleman weighing system (Pori, Finland). The grain yield was adjusted to 14% moisture content following Badu-Apraku et al. (2012) in the Eq.:

$$\text{Grain yield (kg/ha)} = \text{grain yield} \times (100 - \%\ AMC)/(100 - \%\ SMC) \times 100$$

Where: AMC – actual (obtained) grain moisture content (%); SMC – standard moisture content.

Protein, content, and starch. The Pfeuffer Granolyser NIR machine (Pfeuffer, Kitzingen, Germany) was used to analyse the protein and starch contents of the grains. This machine uses NIR diode technology, conducting 1 500 individual scans for each sample. The built-in spectrometer scans the sample seeds within the 950 to 1 540 nm range.

Data analysis. Different N rates and maize cultivars were analysed to assess their effect on agro-physiological parameters, grain yield, and quality. Analysis of variance was conducted after testing the data for normality. Treatment means were compared using least significant difference (*LSD*) and deemed significant if $P < 0.05$. The statistical software Genstat 18^{th} edition (Hemel Hempstead, UK), licensed by Plant Research International, was utilised for the analysis. Furthermore, the relationships between N application rate, maize cultivars, agro-physiological traits, grain yield, and quality were analysed using Pearson's correlation coefficient test in the built-in Excel (Washington, USA) function.

RESULTS AND DISCUSSION

Effects of crop year, nitrogen fertilisation, and cultivar selection on maize yield. Our results showed that significantly applying nitrogen on selected cultivars ($P < 0.05$) enhanced crop yield in the experiment years. The crop yield was lower from 2022 to 2023, irrespective of nitrogen and cultivar treatments (Figure 2); this is expressed by climate variability between the experiment years (Figure 1).

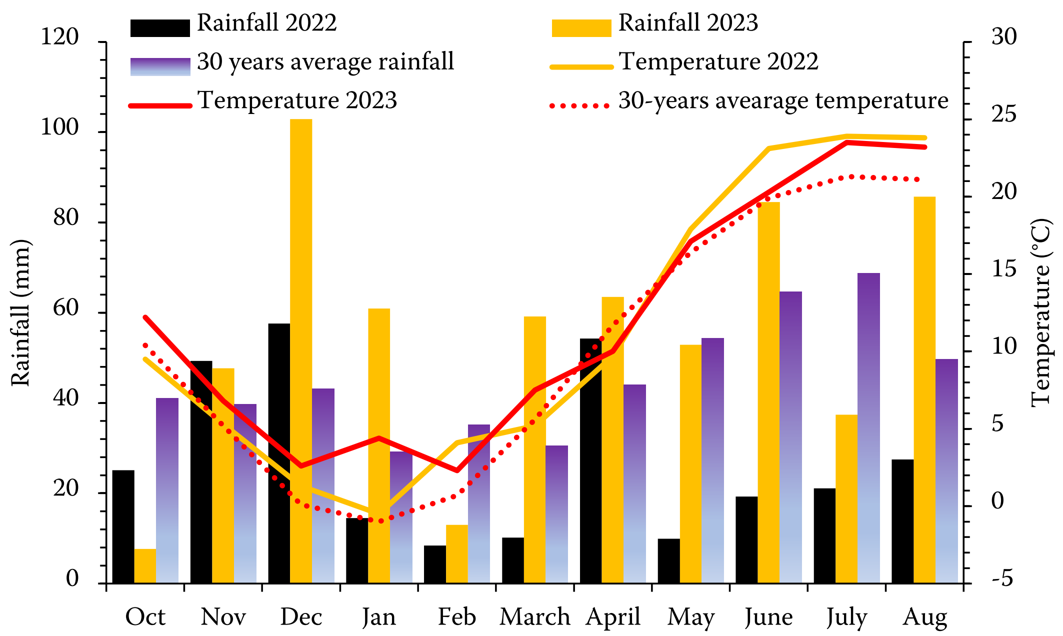

Figure 1. Meteorological data at the Látókép experimental station of the University of Debrecen (2022, 2023)

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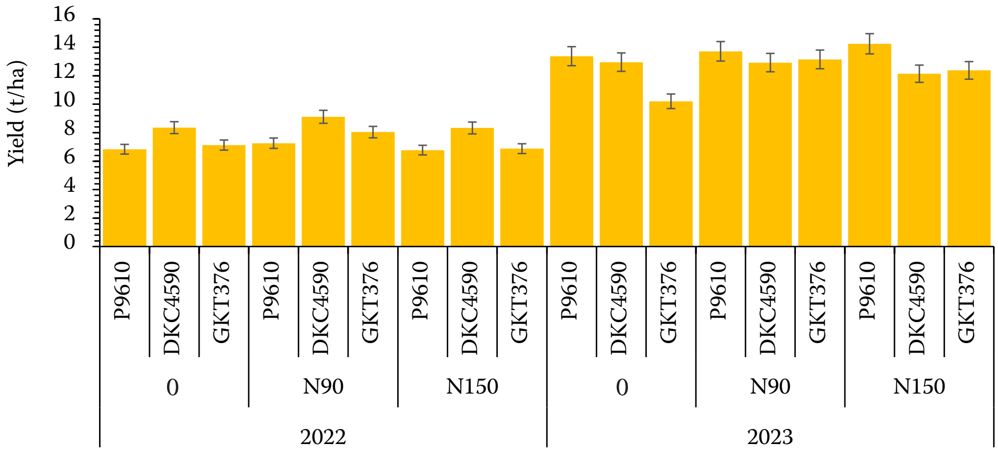

Figure 2. Effects of nitrogen (N) fertilisation on the yield of selected maize cultivars (Debrecen 2022, 2023). Three maize cultivars: P9610-FAO 340, DKC4590-FAO360, and GKT376-FAO360; 0 – 0 kg N/ha; N90 – 90 kg N/ha; N150 – 150 kg N/ha

Yield is affected by interactions between crop year variations, nitrogen fertilisation practices, and cultivar selection strategies (Bojtor et al. 2021). Considering that fertilisation management can bridge the gap of the positive effect of crop year variation on maize yield, evidenced by the superior performance of all cultivars from 2023 to 2022, the efficiency of nitrogen that enables the system to sustain higher yield potential is differentially affected by the maize cultivar. The superior performance of demonstrates this cv. DKC4590 in the unfavourable year 2022; however, it was surpassed by the cv. P9610 in 2023, particularly at the recommended N150 (Bojtor et al. 2021). Therefore, the interaction between well-suited agronomical practices such as nitrogen fertilisation, maturity group, and maize genotypes, which respond to unpredictability in growing season conditions, may provide optimal yields compared to non-interacting traits. In optimising one of these components, understanding all possible interactions and synergistic effects is needed to avoid unintended trade-offs.

This study showed the measurable effects of nitrogen fertilisation and cultivar choice and their interactions on maize grain yield in response to crop year variations. Cultivar response to nitrogen in the years shows GKT376 lacks stability and is highly influenced by nitrogen fertilisation in crop years compared to DKC4590 and P9610. With a similar trend, GKT376 shows the highest performance at N90, where the margins between the control in 2022 and 2023 are 1 t and 3 t, respectively. Therefore, we suggest that adaptive nitrogen fertilisation and cultivar suitability are needed to manage maize production strategies due to unpredictable climatic changes and variations across crop years. Integrated assessments that comprise all components influencing such an adaptation are thus required. Our results substantiate these determinants by considering nitrogen fertilisation practices that depend on cultivar selection. In this scenario, further field experiments on cultivar choice that are authenticated in a more comprehensive and integrated context to assess from an integrated approach and the basis of yield potential with conventional technologies are necessary. Generally, crop year greatly impacted the yield of maize, affecting nitrogen fertilisation regimes and the selected cultivars. This suggests that each crop year has unique conditions that significantly affect the final yield of crops. Following the years' trend, nitrogen fertilisation 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 cultivars was recorded in the interaction of cultivars with N rates – the cv. DKC4590 yielded higher yields, which were recorded at a moderate N rate of N90 than at N150 – the cv. DKC4590 demonstrated superior performance during the year characterised by lower precipitation in 2022, achieving a statistically significant yield of 9.1 t per hectare. In contrast, 2023 did not showcase any notable differences among the cultivars at nitrogen rates of N90 and N150, with GKT376 recording the lowest yield at N0. Therefore, a comprehensive understanding and analysis of the crop year can help farmers make informed decisions regarding the optimal timing and dosage of nitrogen fertilisation and the most suitable cultivars based on available cultivars with informed features.

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Yield response to relative chlorophyll content (SPAD) dynamics in respective cultivars and N rates. The results significantly differed ($P < 0.05$) in chlorophyll content between maize cultivars and N rates associated with the final grain yield. No matter the specific nitrogen rate and crop year, SPAD differences existed among maize cultivars at different growth stages. The chlorophyll content was the highest during the vegetative tasseling (VT) stage and decreased toward the later growth stages (Table 1).

The SPAD index between N rates and cultivars positively influenced yield between years. The decrease 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 vital in the technical evaluation of the photosynthesis capacity (Yan et al. 2021). Therefore, photosynthesis is maximised under optimal leaf chlorophyll content, improving the vegetative structures' dry matter production and intensifying the quantitative points and final yield. Under N rates, the chlorophyll content levels indicate the most adequate plant development stage, indicating the best nitrogen use, directly related to final production. This pigment content can be used as an indicator that adjusts the nitrogen fertilisation levels, using the chlorophyll meter reading technology, allowing for more rapid and real application as an indicative resource of the nitrogen nutritional status in maize and several crops. 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). Therefore, the chlorophyll SPAD index transforms into an accurate, supportive technique, optimising the results in predicting the apparent photosynthesis rate and the final production. Similarly, the crop displayed the lowest and highest SPAD index between growth stages at the V6, R6 and VT stages, respectively. The highest SPAD index was found at the VT stage, followed by the Vn and R6, with no variations, statistically ($P > 0.05$) higher level of SPAD index at VT and R0–R3 than in the V6 and R6 stages (Table 1). However, despite an implicating influence on yield, chlorophyll content indicated a significant ($P < 0.05$) reduction in protein content from 2023 to 2022, respectively.

Effects of N rates and cultivars on NDVI and LAI indices. The NDVI and LAI indices across

Table 1. Chlorophyll content in different growth stages, nitrogen rates and maize cultivars on grain yield in the two experiment years (Debrecen: 2022, 2023)

Source of variation		Growth stage and SPAD					Yield (kg/ha)	Protein	Starch
		V6	VT	R0-R3	R6	AVG		(%)	
Nitrogen fertilisation rates									
	0	44.55	54.82	54.97	47.88	50.55	7 442	9.05	73.19
2022	N90	47.26	57.29	57.02	52.63	53.55	8 141	11.17	69.91
	N150	48.44	58.16	59.26	53.9	54.94	7 330	11.58	71.40
	0	45.56	57.17	54.56	45.71	50.75	12 183	6.73	64.66
2023	N90	44.77	60.1	57.22	45.99	52.02	13 268	7.20	64.52
	N150	48.68	61.28	58.92	46.86	53.94	12 926	7.45	63.84
$LSD_{0.05}$		2.946	2.420	3.000	6.146	2.279	1 443.1	0.5766	2.419
Maize cultivar effects									
	P9610	47.36	58.15	57.53	49.49	53.13	6 960	10.63	72.29
2022	DKC4590	46.69	56.47	56.92	52.44	53.13	8 602	9.79	71.72
	GKT376	46.2	55.66	56.8	52.48	52.78	7 351	11.38	70.48
	P9610	48.7	59.74	55.92	44.91	52.32	13 784	7.27	64.32
2023	DKC4590	44.85	57.86	55.27	45.66	50.91	12 679	6.82	64.94
	GKT376	45.45	60.95	59.52	48.01	53.48	11 914	7.30	63.76
$LSD_{0.05}$		2.994	2.117	1.949	2.820	1.022	959.7	0.464	2.055

NB – V6; VT – vegetative phases; R0–R3 – reproductive phases; R6 – reproductive phase; AVG – average SPAD per N rate and cultivar; 0 – 0 kg N/ha; N90 – 90 kg N/ha; N150 – 150 kg N/ha; *LSD* – least significant difference

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various growth stages were significant ($P < 0.05$) within cultivars and N rates in the years. The growth stage displayed considerable effects on NDVI and LAI values, demonstrated by a steady increase as the crop advanced through growth stages, reaching their lowest point at the V6 and R6 stage and peaking at the VT and R0–R3 stages with the crop year interaction (Figures 3 and 4).

Leaf area index and normalised difference vegetation index have been widely used for assessing crop health and potential yield in various contexts (Belmahi et al. 2023). It is essential to explore the underlying interrelationship to interpret respective plant processes since they are vital predictors in maize yield potential assessment in growth stages. In 2022, LAI ranged from 0.9 to 4.0 and NDVI from 0.4 to 0.73, while in 2023, values ranged from 2.13 to 3.93 for LAI and 0.408 to 0.81 for NDVI. The highest NDVI was recorded in the VT, followed by R0. This aligns with Támas et al. (2023), suggesting that earlier stages can predict yield, contrasting with Yang et al. (2022), suggesting R3.

Conversely, the LAI values showing the maximum values in 2022 indicated the low yield in the 2022 to 2023 crop year. This could be influenced by the genetic landscape of the cultivars as affected by the crop year. 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. The findings imply that NDVI and LAI data could help analyse the ecological performance of plant growth dynamics in the field and the final crop yield and quality, thereby contributing to the development of innovative farming technologies.

Relationship between grain yield, protein, starch and physiological parameters. Correlation analysis was significant to determine the relationship between grain yield and quality and the various agrophysiological parameters ($P < 0.05$). 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. It is well known that cultivar varieties and mineral nutrition significantly affect maize yield (Bojtor et al. 2022). In our study, all the examined cultivar varieties showed a distinctive positive correlation between the chlorophyll content and yield during the vegetative stages. Another positive correlation observed was between chlorophyll content and protein. These observations indicate that chlorophyll content is essential in determining the quantity and quality of maize production. The chlorophyll content can be increased by downstream elements that promote maize vegetative growth (Yang et al. 2023). In this study, the significant impact of nitrogen levels is evidenced on all the examined traits. The indices SPAD, LAI, and NDVI measured at the vegetative tasseling stage (VT) and the reproductive stages R0–R3 exhibit a strong and positive correlation with

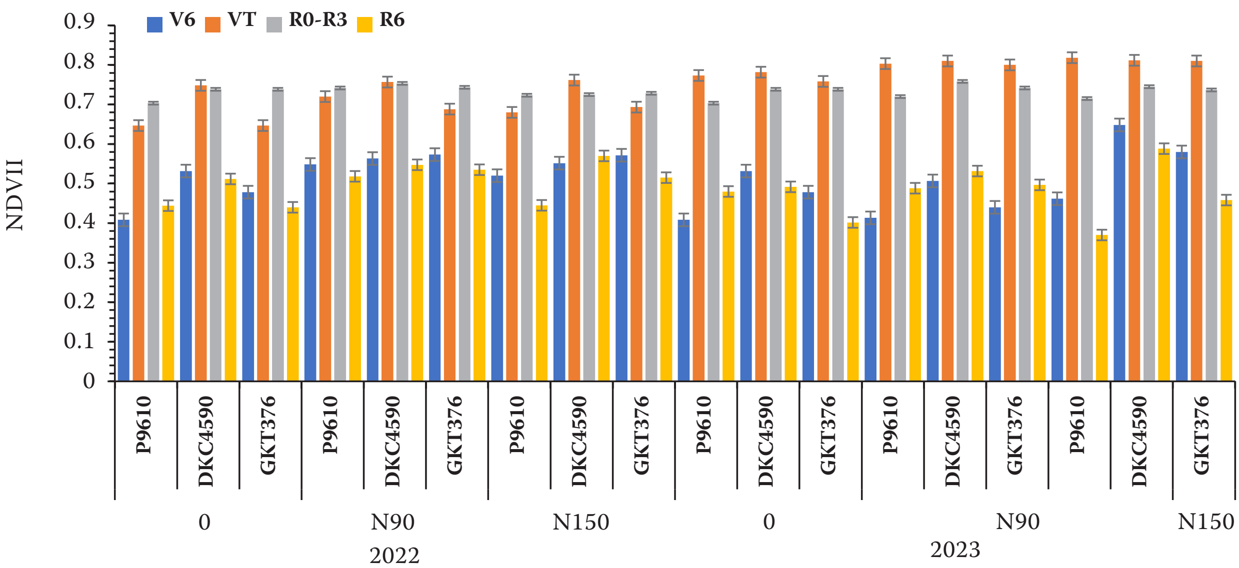

Figure 3. Interactive effects of nitrogen (N) rates, cultivar genotypes, and developmental stages on normalised differences vegetation index (NDVI) (Debrecen: 2022, 2023). NB – V6; VT – vegetative phases; R0–R3 – reproductive phases; R6 – reproductive phase

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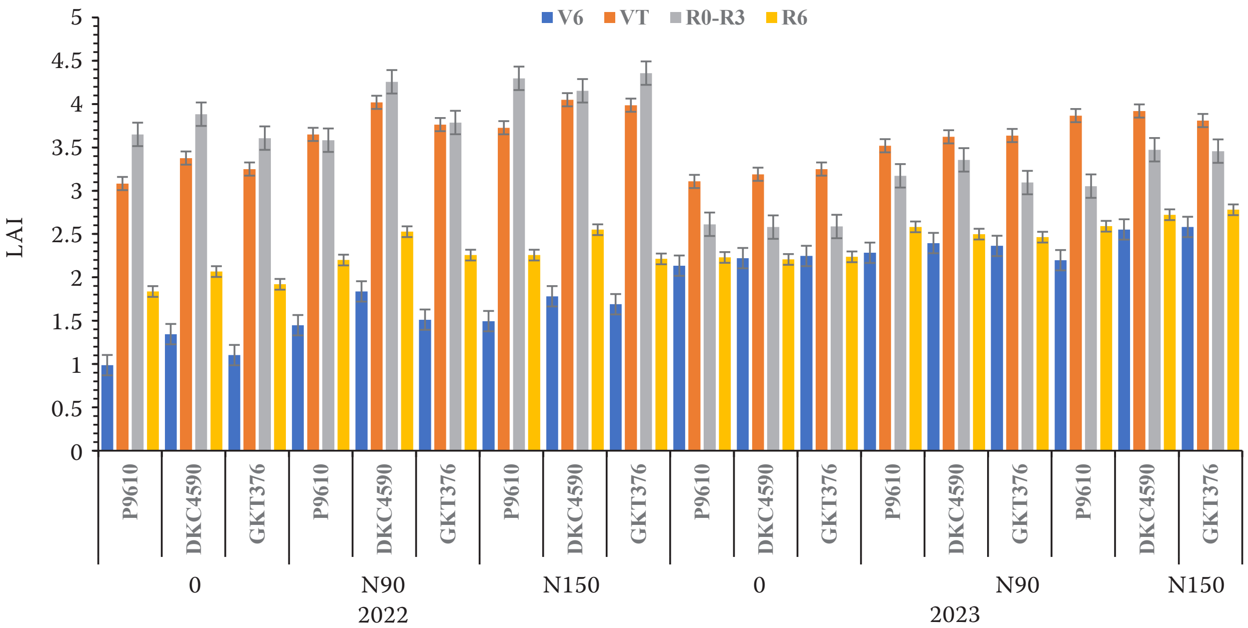

Figure 4. Interactive effects of nitrogen (N) rates, cultivar genotypes, and developmental stages on leaf area index (LAI) (Debrecen: 2022, 2023). NB – Vn; VT – vegetative phases; R0–R5 – reproductive phases; R6 – reproductive phase; 0 – 0 kg N/ha; N90 – 90 kg N/ha; N150 – 150 kg N/ha

the overall crop yield. Thus, when suitable and appropriate cultivars 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 optimise yield outcomes in agricultural practices. Regarding quality, the indices positively and strongly correlate with protein, particularly during VT and R0–R3 stages, indicating the usability of the parameters at

Table 2. Pearson correlation coefficients: physiological parameters, grain yield, and protein and starch (Debrecen: 2022, 2023)

	Yield (kg/ha)	Protein (%)	Starch (%)	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
Yield (kg/ha)	1.00														
Protein (%)	0.34	1.00													
Starch (%)	0.15	–0.85	1.00												
SPAD-V6	0.61	0.85	–0.62	1.00											
SPAD-VT	0.50	0.81	–0.54	0.88	1.00										
SPAD: R0-R5	–0.01	0.92	–0.90	0.62	0.69	1.00									
SPAD-R6	–0.67	0.44	–0.74	0.03	0.20	0.75	1.00								
LAI-V6	–0.24	0.43	–0.40	0.21	0.61	0.65	0.68	1.00							
LAI-VT	0.22	0.98	–0.85	0.81	0.85	0.96	0.56	0.61	1.00						
LAI: R0-R3	0.31	0.70	–0.42	0.62	0.90	0.72	0.36	0.84	0.80	1.00					
LAI-R6	0.34	0.81	–0.55	0.76	0.96	0.78	0.37	0.77	0.88	0.98	1.00				
NDVI-V6	0.43	–0.25	0.19	–0.26	0.18	–0.01	0.32	0.74	–0.03	0.42	0.30	1.00			
NDVI-VT	0.56	0.65	–0.24	0.69	0.91	0.55	0.05	0.65	0.70	0.95	0.93	0.29	1.00		
NDVI:R0-R3	0.22	0.96	–0.80	0.72	0.81	0.97	0.58	0.65	0.98	0.83	0.88	–0.01	0.73	1.00	
NDVI-R6	0.07	–0.38	0.63	–0.36	0.05	–0.28	–0.19	0.45	–0.25	0.35	0.17	0.72	0.40	–0.15	1.00

–1.0 –0.75 –0.5 –0.25 0 0.25 0.5 0.75 1.0

SPAD – relative chlorophyll content; LAI – leaf area index; NDVI – normalised differences vegetation index; NB – Vn; VT – vegetative phases; R0–R5 – reproductive phases; R6 – reproductive phase

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the specific growth stages in predicting the grain quality of maize.

The parameters SPAD, NDVI and LAI indicated a general positive correlation with grain protein content, while a negative correlation with starch (Table 2). This relationship with proteins aligns with numerous studies recording negative correlation between protein and starch and antagonistic in some exceptions, like water logging (Melash et al. 2023). The amelioration of protein has been linked with subjecting crops to drought stress (Javed et al. 2022), which is attributed to the drought conditions in 2022. Thus, the improved grain protein under drought conditions in 2022 could be due to the reduction in grain starch accumulation, and the limited starch accumulation allows for a higher concentration of nitrogen per unit of starch in the grains (Melash et al. 2023). As a result, the decreased starch is attributed to loose packaging in the starch granules, possibly due to decreased amylose composition (Prathap et al. 2019). Subsequently, drought conditions 2022 resulted in early senescence shortening the grain filling stage, reducing starch accumulation (Prathap et al. 2019). Additionally, early senescence in crops limits starch accumulation by influencing crops' photosynthetic capacity, reducing yield as recorded in 2022. The results reflect a critical correlation between the non-destructive indicators SPAD, NDVI, and LAI in terms of the yield of maize and the content of the essential quality parameters of starch and grain protein. Our results showed that higher non-destructive indices, particularly SPAD and NDVI values during the growing season, indicated higher grain yield and influenced grain protein and starch contents.

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