HYPERSPECTRAL IMAGERY FOR PREDICTION OF LEAF CHLOROPHYLL CONTENT IN MAIZE UNDER THE APPLICATION OF DIFFERENT UREASE INHIBITORS USING MACHINE LEARNING

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Abstract.

Urea is the most common and widely used nitrogen (N) source. However, it is highly susceptible to ammonia volatilization losses, especially under favorable climatic conditions. The use of urease inhibitors becomes an important strategy because these compounds slow down the hydrolysis of urea, increasing efficiency in terms of N assimilation, enhancing leaf chlorophyll content, promoting plant growth, and maximizing maize grain yield. In parallel, hyperspectral sensors have emerged as a non-destructive alternative to conventional methods for assessing plant nutritional status. This study aimed to identify and analyze hyperspectral signatures associated with different urea sources and nitrogen application rates in maize leaves, and to relate these signatures to leaf chlorophyll content using machine learning. The experiment was conducted under field conditions at the Agronomic Experimental Station of UFRGS (Federal University of Rio Grande do Sul), in Eldorado do Sul, southern Brazil, using the hybrid DKB 358 sown on October 23rd, 2025, in a randomized block design with four replications, totaling 40 experimental plots. Treatments consisted of three N rates (90, 180 and 360 kg N ha^{-1}) combined with different nitrogen sources (conventional urea and two commercial urease inhibitors) and a control (without N). The last fully expanded leaf from each plant was sampled, and hyperspectral scanning (400 – 1000 nm) using the Pika L® imaging system (Resonon Inc., MT, USA) was performed. Leaf chlorophyll content was measured with a portable chlorophyll meter (Falker® CFL 1030) (Falker Automoção Agrícola Ltda) at four different points on the leaf blade. Data were collected at two phenological stages (V13 and R1- flowering) and the Partial Least Squares Regression (PLSR) algorithm was applied to the dataset to train (70%) and test (30%) the leaf chlorophyll estimation model. The performance of the model was assessed using the best value of the coefficient of determination (R^2) and the root mean square error (RMSE). Treatments containing urease inhibitors resulted in higher leaf chlorophyll levels, indicating improved nitrogen assimilation. The PLSR model exhibited high predictive accuracy for leaf chlorophyll content for the vegetative growth stage V13 (R^2 = 0.87; RMSE = 2.6) and flowering R1 (R^2 = 0.91; RMSE = 2.4). The Variable Importance in Projection (VIP) scores analysis identified the spectral regions most associated with leaf chlorophyll variation, corresponding to red region (600.36 nm) and red-edge region (738.79 nm). Hyperspectral imaging is an effective non-destructive tool for monitoring variations in nitrogen status and leaf chlorophyll content, supporting more precise and efficient fertilizer use in maize.

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Keywords.
Spectral signatures, Non-destructive sensing, Nitrogen fertilization, Precision agriculture

Introduction

Urea is widely used as a nitrogen (N) source in maize cultivation; however, it is subject to losses through ammonia volatilization (Pan et al. 2016). Urease inhibitors are used to reduce these losses, increasing nitrogen use efficiency (Cantarella et al. 2018).
Leaf chlorophyll content (LCC) is directly related to the nitrogen nutritional status of plants and is widely used as a physiological indicator (Schlichting et al. 2015). Unlike multispectral approaches, hyperspectral data provide continuous spectral information across a wide range of wavelengths, enabling the detection of subtle variations in leaf-level reflectance associated with plant physiological traits (Panda et al. 2025). Machine learning techniques such as Partial Least Squares Regression (PLSR) enable the identification of informative spectral regions and the development of robust predictive models for chlorophyll- and nitrogen-related traits at LCC and canopy chlorophyll content (CCC) scales from hyperspectral data (Yendrek et al. 2017; Wang et al. 2021; Kanning et al. 2018). Thus, this study aimed to identify and analyze hyperspectral signatures associated with different urea sources and nitrogen application rates in maize leaves and to relate these signatures to LCC using machine learning.

Material and Methods

The experiment was conducted in the Experimental Agronomic Station of the Federal University of Rio Grande do Sul, located in the municipality of Eldorado do Sul, southern Brazil (30°06'39.3"S 51°40'51.5"W) (Fig. 1).

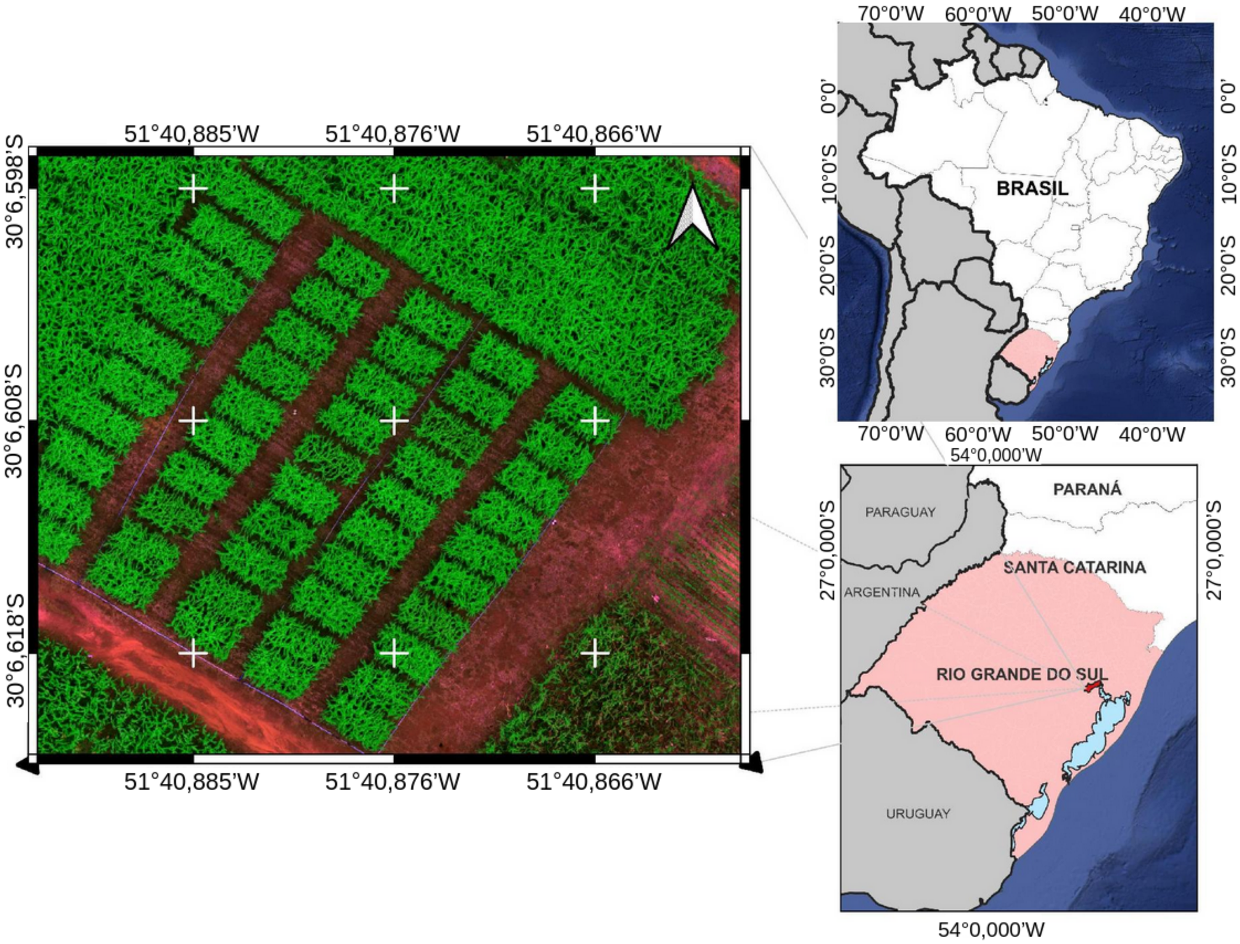

Figure 1. Area 11 of the Federal University of Rio Grande do Sul Experimental Agronomic Station (left), located in Eldorado do Sul, Rio Grande do Sul (right), southern Brazil.

A randomized block design with four replications was adopted, totaling 40 experimental plots. The treatments consisted of nitrogen (N) topdressing rates distributed into two main ranges (90 kg N/ha and 180 kg N/ha) and one complementary range with phosphorus (P) treated with rates of 180 kg N/ha and 360 kg N/ha. In the main ranges, six treatments were evaluated: white urea, 3 kg Volit, 2 kg Volit, 2.5 kg Prokit, commercial treatment, and no N application (control). In the range with treated P, only the 3 kg Volit treatment was evaluated at two rates (180 kg and 360 kg N/ha).
Sowing was carried out on 10/23/2025 at the Experimental Agronomic Station (EEA), using the maize variety DKB 358, sown in five rows with 0.5 m spacing. After emergence, the plots were delimited to an area of 5 m², maintaining a population of 4 plants/m² (approximately 100 plants per plot).
Hyperspectral data were acquired in a dark room under controlled temperature conditions (25 °C), using artificial illumination provided by halogen lamps mounted on a tower, at the Plant Physiology Laboratory of the Department of Field Crops, Faculty of Agronomy, at the Federal University of Rio Grande do Sul. The acquisition system consisted of a push-broom hyperspectral camera (PIKA L, Resonon Inc®) equipped with a 23 mm objective lens, positioned 30 cm above the scanning platform containing the maize leaves (Fig. 2). The camera recorded data within the spectral range from 400 to 1000 nm, distributed across 281 bands (spectral channels), with a spectral resolution of 3.1 nm, bandwidth of 2.7 nm, and radiometric resolution of 12 bits. Each captured line contained 900 spatial pixels. Although the maximum acquisition rate is 249 fps, a rate of 21 fps was used in this

study. Two-dimensional images were generated by combining line-by-line scans as the sample moved relative to the camera using a linear translation stage (Fig. 2).

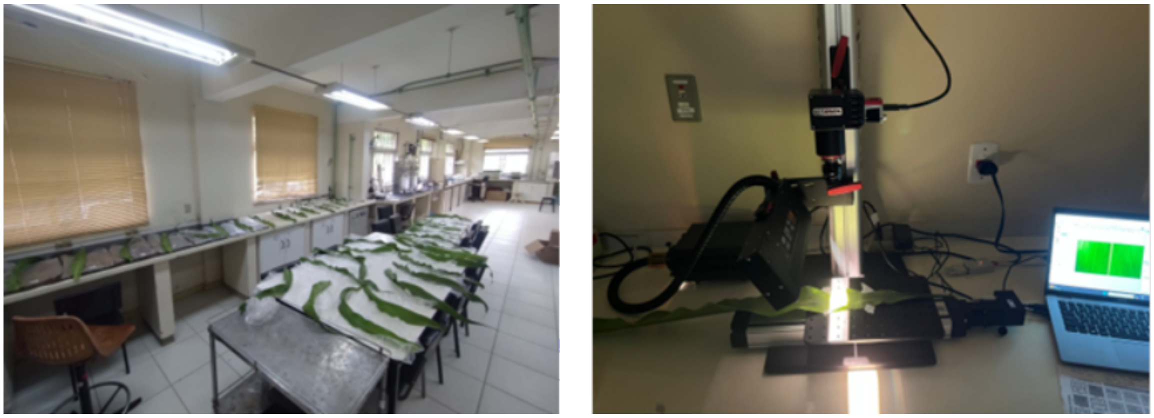

Figure 2. Preparation and acquisition of hyperspectral images: (a) maize leaves obtained after field collection; (b) hyperspectral sensor (Pika L, Resonon Inc®) used

White calibration was performed using a polyethylene reference panel (Spectronon Pro Resonon®), while dark calibration was conducted by covering the lens with a non-reflective black cap, ensuring standardization compensates for variations in illumination conditions (Fig. 2).

A representative leaf was collected from each treatment plot, totaling 40 samples. The selected leaf corresponded to the last fully expanded leaf of each plant. The samples were placed in plastic bags and stored in a cooled thermal box containing reusable ice packs (Gelox) for transport and subsequent hyperspectral camera analysis (Fig. 2).

Measurements were performed in four pre-selected areas on the adaxial surface of the leaves, with two measurements taken from the upper third and two from the middle third of the leaf, excluding the basal region due to the greater influence of the central vein area (Fig. 2).

After hyperspectral measurements, chlorophyll parameters were collected using the Falker® CFL 1030 chlorophyll meter, which measures transmittance at wavelengths of 635 nm, 660 nm, and 880 nm, using calibration based on a white reference plate. From the transmittance measurements obtained with the ClorofiLOG (Falker, Porto Alegre, Brazil), chlorophyll a (*Chl* a) and chlorophyll b (*Chl* b) parameters, as well as their sum (*Chl* a+ *Chl* b), corresponding to total chlorophyll, were calculated based on the Falker index.

Prior to model development, hyperspectral images were converted to reflectance using white and dark reference calibration. The spectral dataset was then preprocessed before entering the machine learning algorithm. The original spectral information was subjected to spectral trimming, Standard Normal Variate (SNV) normalization, and Savitzky–Golay filtering with first derivative transformation. Thus, the PLSR models were not fitted using raw reflectance spectra but using preprocessed derivative spectral variables. For each leaf sample, chlorophyll readings were organized according to leaf position: the first two measurements were considered from the middle third of the leaf and the following two from the upper third. The mean value of the two measurements from each leaf section was calculated separately, and the chlorophyll response variable was assigned according to the evaluated leaf section.

These bands were used as candidate predictors, and an exhaustive combination search tested subsets from three bands up to all selected bands. The combination showing the best predictive performance was selected for subsequent model development and evaluation.

Partial Least Squares Regression (PLSR) was then applied to estimate total leaf chlorophyll content from the selected preprocessed spectral bands. The dataset was divided into training and testing subsets, with 70% of the samples used for model calibration and 30% for testing. The PLSR model was fitted using three latent variables, with internal scaling of the predictor variables enabled during model training. Model performance was evaluated using the coefficient of determination (R^2), root mean square error (RMSE), mean absolute error (MAE), and mean squared error (MSE). The relative contribution of each wavelength was assessed using Variable Importance in Projection (VIP) scores, adopting VIP > 1.0 as the threshold for identifying the most relevant bands. In addition, regression coefficients from the best PLSR model were exported and analyzed to evaluate the magnitude and direction of the contribution of each selected wavelength to chlorophyll prediction. Processing was done in Python, using library packages of pandas, numpy, matplotlib, seaborn, scikit-learn, imbalanced-learn, scipy and statsmodels.

Results and Discussion

Our results showed that treatments containing urease inhibitors resulted in higher LCC. Similarly, Cezar et al. (2024) reported increased chlorophyll levels in maize plants fertilized with urea combined with a urease inhibitor. This response is likely associated with reduced nitrogen losses through ammonia volatilization, leading to greater nitrogen availability for plant uptake and, consequently, enhanced nitrogen assimilation and chlorophyll biosynthesis.

The PLSR model demonstrated high predictive accuracy for LCC at both the vegetative stage V13, with coefficient of determination (R^2) of 0.87 and a root mean square error (RMSE) of 2.6 (Fig. 3a), and the flowering stage R1, with R^2 of 0.91 and RMSE of 2.4 (Fig. 3b). These results indicate that hyperspectral reflectance combined with PLSR was effective for estimating leaf chlorophyll content in maize, particularly at R1.

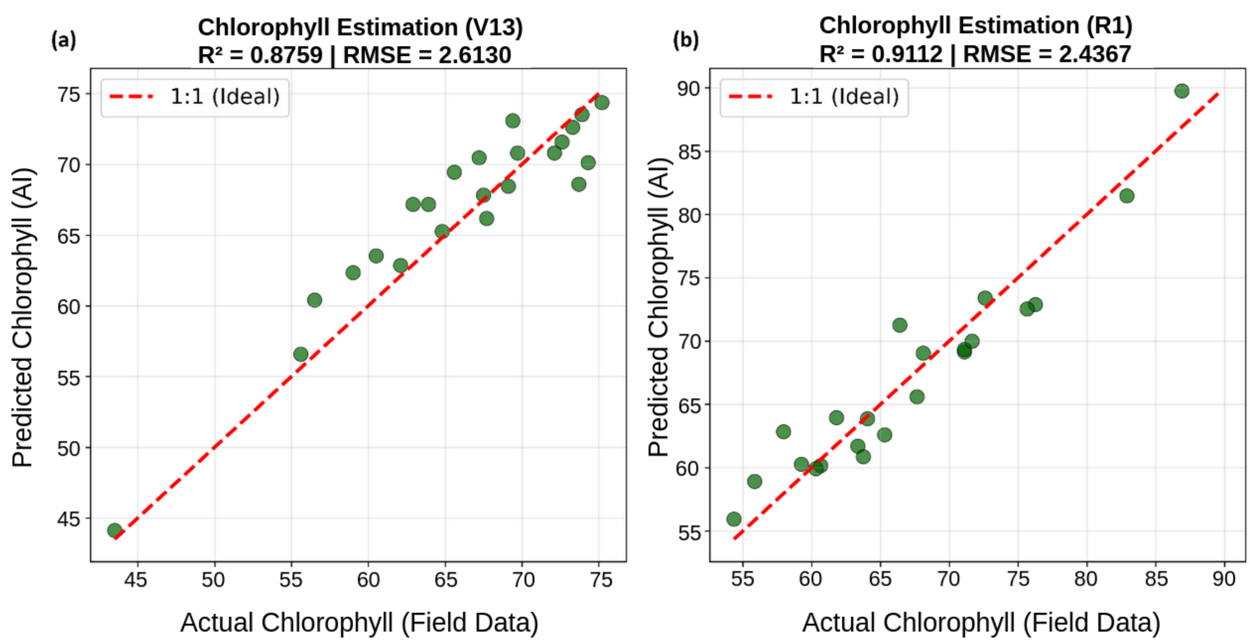

Figure 3. Predictions performance of the PLSR model for chlorophyll content across two acquisition dates: (a) V13 vegetative stage and (b) R1-flowering stage.

As phenological development progresses and symptoms of nutrient deficiency become visible due to the internal remobilization of nutrients within the plant, the relationship between spectral reflectance and leaf chlorophyll content (LCC) exhibits more distinct spectral signatures. This phenological maturation increases spectral variability within the dataset, enhances class discrimination, and improves the predictive performance of the models, resulting in higher R^2 values at the later stages (Yang et al. 2022; Chen et al. 2023).

Analysis of the Variable Importance in Projection (VIP) scores revealed stage-dependent spectral contributions to chlorophyll prediction. At the vegetative stage (V13), the wavelengths with VIP scores above 1.0 were 659.54 nm and 600.36 nm (Fig. 4a), indicating that visible wavelengths in the orange-red and red region were the main drivers of the PLSR model at this stage. This response is consistent with the sensitivity of leaf reflectance to chlorophyll variation in the 530-630 nm interval (Gitelson and Merzlyak 1998) and with chlorophyll absorption in the red region, although wavelengths around 660-680 nm may become less sensitive under higher chlorophyll concentrations due to saturation effects (Wu et al. 2008).

At the flowering stage (R1), the most important wavelengths were 738.79 nm and 600.36 nm, both with VIP scores above 1.0 (Fig. 4b). The importance of wavelengths near 600.36 and 738.79 nm observed in this study can be attributed to the strong absorption of chlorophyll pigments in the visible spectrum and the high sensitivity of the red-edge region to changes in chlorophyll concentration (Li et al. 2025). The persistence of 600.36 nm in both stages suggests that this wavelength represents a stable spectral feature associated with chlorophyll variation across maize development.

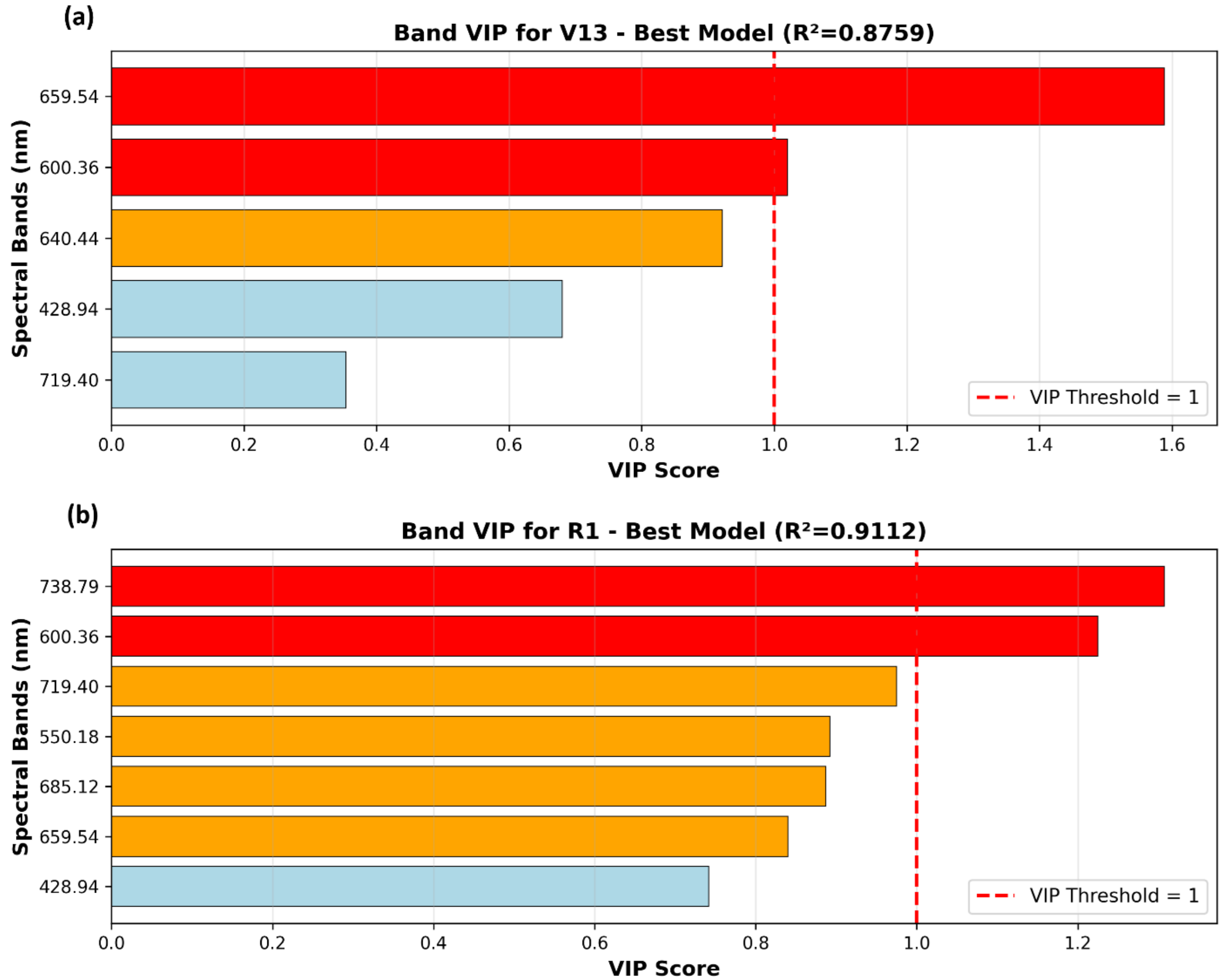

Figure 4. Variable Importance in Projection (VIP) scores of the spectral bands selected by the best PLSR models for leaf chlorophyll prediction in maize at the (a) vegetative stage V13 and (b) reproductive stage R1.

Overall, these results indicate that chlorophyll prediction in maize is strongly dependent on the phenological stage. During V13, model performance was primarily driven by wavelengths in the visible red region, whereas at R1 the red-edge region became the dominant contributor to chlorophyll estimation. This spectral shift is likely associated with changes in leaf age, canopy architecture, nitrogen remobilization, and pigment dynamics occurring during the transition from vegetative growth to flowering (Yang et al. 2022). The spectral features associated with maize leaf chlorophyll content varied across growth stages, with improved model performance at later developmental stages and significant contributions from red-edge-related bands (Guo et al. 2024). Furthermore, Sudu et al. (2022) reported that UAV-based hyperspectral models for chlorophyll content estimation in summer maize selected key wavelengths within the visible and red-edge regions, including bands near 550–566 nm and 706–720 nm.

These findings reinforce the importance of considering crop phenology when developing spectral models for chlorophyll estimation and support the relevance of visible and red-edge wavelengths as robust indicators of chlorophyll dynamics throughout maize development.

Conclusion

This study demonstrated that leaf-level hyperspectral imaging combined with PLSR is a promising approach for estimating maize chlorophyll content and detecting nitrogen nutritional status in maize. The controlled acquisition protocol, including standardized illumination, white and dark calibration, fixed sensor geometry, and measurements on defined leaf regions, provided consistent spectral data for model development. The high predictive performance observed at both V13 and, especially, R1 indicates that hyperspectral data can effectively capture chlorophyll-related spectral variation during maize development.

The use of VIP scores allowed the identification of the most informative wavelengths, reducing the dimensionality of the hyperspectral dataset while improving model interpretability. This approach may support the future development of more simplified spectral models focused on key wavelengths, with potential application in proximal, UAV-based, or orbital sensing platforms. Although further validation under field and canopy-scale conditions is required, the proposed approach shows strong potential for monitoring large agricultural areas and may be adapted to other crops, contributing to more efficient nutritional diagnosis and precision nitrogen management.

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