Wavelength-Selective Partial Least Squares Regression of Near-Infrared Spectra for Predicting Chlorophylls and Carotenoids in Leaves

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Abstract*: *This study investigates the quantification of leaf pigments using near-infrared (NIR) spectroscopy, with an emphasis on the application of wavelength-selective partial least squares regression (WSPLSR) for improved predictive modelling. While conventional PLSR yields varying prediction accuracies—chlorophyll a (R^2 = 0.922), chlorophyll b (R^2 = 0.853), chlorophyll a+b (R^2 = 0.910), and carotenoids (R^2 = 0.787)—the incorporation of wavelength selection significantly enhances model performance. The WSPLSR approach identifies optimal spectral regions strongly correlated with pigment concentrations, achieving higher coefficients of determination: chlorophyll a (R^2 = 0.978), chlorophyll b (R^2 = 0.953), chlorophyll a+b (R^2 = 0.974), and carotenoids (R^2 = 0.908). Model robustness is confirmed by the corresponding RPD values: chlorophyll a (RPD = 6.761), chlorophyll b (RPD = 4.593), chlorophyll a+b (RPD = 6.2), and carotenoids (RPD = 3.29). These findings demonstrate the efficacy of WSPLSR models in NIR spectroscopy as a rapid, non-destructive, and non-invasive tool for farmers, facilitating the early detection and management of pigment-related stress in plants.

***Keywords*: NIR Spectroscopy, Wavelength-Selective PLSR, Plant Pigment Prediction, Non-Destructive Leaf Analysis.**

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I. INTRODUCTION

Chlorophyll (Chl), a primary pigment found in all green plants, is essential for photosynthesis as it absorbs sunlight and utilises this energy to convert water, carbon dioxide, and minerals into oxygen and energy-rich organic compounds such as carbohydrates [1]. A lack of Chl in plants can cause yellowing or pale leaves, indicating a deficiency of this vital pigment necessary for photosynthesis. Synthesis of Chl depends on various nutrients, such as nitrogen, magnesium, and iron. A shortage of these nutrients can lead to Chl deficiency [2]. Chl *a* is utilised in oxygenic photosynthesis, capturing energy from blue and red bands while showing limited absorption of the green band of the spectrum. Chl *a* molecules are involved in the transfer of electrons, essential for generating ATP and NADPH, the energy carriers used in the subsequent synthesis of organic molecules [3]. Chl *b* is also used in photosynthesis, absorbing light energy in the blue band. It assists in photoprotection by dissipating excess light energy as heat, thus preventing damage to the photosynthetic mechanism [4].

Carotenoids (Car) are crucial for capturing light energy and transferring it to Chl molecules, the primary pigments responsible for photosynthesis. They are essential for photoprotection, especially under stress conditions such as excess light, high temperatures, or drought. Car serves as antioxidants, scavenging harmful reactive oxygen species that can be generated during photosynthesis in the presence of excess light [5, 6]. By neutralising reactive oxygen species, Car prevents damage to the cellular structure, enhancing the ability of plants to tolerate environmental stress [7]. Car acts as accessory pigments in photosynthesis, facilitating light capture and energy transfer, while also serving as antioxidants, protecting cells from damage caused by excess light and oxidative stress [8].

Spectrophotometry and fluorometry, both established techniques, measure the light absorption by Chl in leaves. Solvents such as acetone, methanol, or ethanol extract Chl pigments, aiding in the breakdown of cell membranes and the release of pigments [9]. After centrifugation, the Chl concentration is determined through spectrophotometric readings at specific wavelengths, as Chl absorbs light in the blue and red bands. Fluorometry uses blue or red excitation sources, detecting Chl fluorescence primarily in the red region [10]. High-Performance Liquid Chromatography (HPLC) is another technique that isolates and measures individual Car in plant extracts precisely [11]. Thin-layer chromatography is an economical alternative, allowing for Car separation via capillary action and visualisation under ultraviolet light [12]. Liquid chromatography-mass spectrometry and gas chromatography-mass spectrometry are other techniques that quantify Car based on mass-to-charge ratios, while colourimetric assays determine Car content, although these techniques are expensive and time-consuming. Skilled technicians are required for the operation of these techniques [13].

TABLE I. COMPARISON OF SPECTROSCOPIC TECHNIQUES FOR THE CHARACTERIZATION OF CHLOROPHYLL AND CAROTENOIDS IN PLANT LEAVES.

Spectroscopic Technique	Principle	Detection of Chlorophyll	Detection of Carotenoids	Advantages	Limitations
UV–Visible Spectroscopy	Measures light absorption due to electronic transitions	Strong absorption bands (430 and 662 nm)	Absorption in 400–500 nm region	Simple, rapid, quantitative	Limited specificity; overlapping bands; often requires extraction
Fluorescence Spectroscopy	Measures emitted light after excitation	Highly sensitive chlorophyll fluorescence (685 and 735 nm)	Weak or negligible fluorescence	Very sensitive; widely used in plant physiology	Carotenoids poorly detected; fluorescence quenching effects
Raman Spectroscopy	Inelastic scattering of monochromatic light	Characteristic porphyrin ring vibrations	Strong resonance-enhanced C=C and C–C bands	Non-destructive; molecular specificity; in situ detection	Fluorescence background; lower sensitivity for chlorophyll
FTIR Spectroscopy	Measures vibrational absorption of IR radiation	Indirect detection via functional groups	Indirect detection via C–H and C=C vibrations	Label-free; provides biochemical context	Low specificity; water interference; limited pigment selectivity
NIR Spectroscopy	Overtone and combination vibrational modes	Indirect correlation with chlorophyll content	Indirect estimation via chemometrics	Rapid; non-destructive; field-deployable	Requires calibration models; indirect measurement
Hyperspectral Imaging	Combines spectroscopy with spatial imaging	High sensitivity via reflectance indices (e.g., NDVI)	Detected through spectral signatures	Spatial mapping; remote sensing capability	High cost; complex data analysis; indirect pigment assignment

Optical spectroscopy is a modern tool used for the rapid and non-destructive evaluation of various biochemicals present in plants, as summarized in Table 1 [14]. Artificial intelligence plays a significant role in precision agriculture, empowering farmers or agricultural engineers to make data-driven decisions and optimise various aspects of crop cultivation [15]. Machine learning algorithms rely on spectral features, such as absorbance, transmittance, and reflectance values at particular wavelengths associated with unique chemical bonds or functional groups. Artificial neural networks and partial least squares (PLS) regression (PLSR) models, when applied to visible-near-infrared (NIR) spectra, demonstrate higher correlation coefficients of 0.92 and 0.97, respectively, for predicting Chl presence in winter wheat within the spectral range of 350-1400 nm. PLSR models applied to reflectance visible-NIR spectra within 500-1039 nm demonstrate enhanced predictive capability, yielding a coefficient of determination (R^2) of 0.80 for Chl presence [16]. PLS analysis of twelve visible-NIR reflectance indices detects Chl content associated with varied nitrogen levels in rice leaves, achieving an impressive R^2 value of 97.8%. Cross-validated PLSR models of NIR spectra establish correlations with the physiological attributes of leaves, achieving a remarkable 72% accuracy in predicting Chl content within the spectral range of 1100-2500 nm [17]. NIR spectroscopy was employed to determine the Car content in maize in correlation with the findings derived from HPLC analysis of the extracts [18]. Predictive models estimate Car, lutein, and β-carotene contents in the skin and flesh of summer squash fruits, achieving a maximal R^2 value of 0.96 [19, 20].

The significance of this study lies in its methodological advancement in estimating leaf pigment concentrations—specifically chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), total chlorophyll (Chl *a+b*), and carotenoids (Car)—by comparing the predictive performance of wavelength-selective partial least squares regression (WSPLSR) models with that of conventional PLSR models. Traditional pigment quantification methods, such as spectrophotometric analysis, involve chemical extraction and are inherently destructive, time-consuming, and impractical for rapid or large-scale applications. NIR spectroscopy offers a non-destructive, fast, and scalable alternative. This study utilises NIR spectral data alongside reference pigment concentrations obtained through standard spectrophotometry to evaluate and compare the performance of WSPLSR and ordinary PLSR models. Robust model generalisation across diverse leaf samples is essential for developing practical, field-deployable diagnostic tools applicable to a wide range of crops and varying agronomic conditions. Accurate and rapid prediction of pigment deficiencies facilitates early detection of physiological stress or nutrient imbalances, enabling timely and targeted agronomic interventions. By demonstrating the superior performance of WSPLSR over conventional PLSR, this study establishes a methodological foundation for the development of rapid, non-invasive, and scalable tools for in-field plant pigment assessment, thereby supporting data-driven decision-making in modern precision agriculture.

II. MATERIALS AND METHODS

A. Dataset

The spectral dataset utilised in this study was collected from the Ecological Spectral Information System (EcoSIS) library. It consists of NIR spectra acquired from temperate plants grown at the National Institute of Agricultural Research (INRA) in Angers, France, in 2003. Winters are mild, with occasional snowfall, while summers are warm and pleasant, occasionally accompanied by heatwaves. Spring and autumn bring moderate temperatures and moderate rainfall.

Spectra were measured using a portable ASD FieldSpec spectroradiometer within the wavelength range of 400 to 2400 nm, with spectral sampling of 1.4 nm and 2 nm for the ranges of 400-1050 nm and 1000-2400 nm, respectively. Two hundred sixty-four reflectance spectra were captured from a diverse selection of forty-three dicotyledonous plants. Some distorted spectra were eliminated from the dataset for better preprocessing.

Two preprocessing methods, Multiplicative Scatter Correction (MSC) and Savitzky-Golay (SG) finite impulse response (FIR), were applied to eliminate noise in the NIR spectra, as illustrated in Fig. 1. MSC was applied to the raw NIR spectral data to eliminate multiplicative effects arising from photon scattering, utilising MATLAB 2023b. It is performed by fitting each spectrum to a reference spectrum using a simple linear regression that estimates an additive offset and a multiplicative scaling factor. The spectrum is then corrected by subtracting the estimated offset and dividing by the estimated scaling factor, thereby reducing scattering-induced baseline shifts and intensity variations. An SG filter with an order of 2 and a frame length of 7 was applied for ordinary PLSR. A filter with an order of 8 and a frame length of 15 was used for WSPLSR to enhance the smoothing of noisy first derivative spectra with higher frequencies, utilising Google Colab. The SG spectral smoothing is performed by fitting a low-order polynomial to a small moving window of adjacent spectral points using least-squares minimization. The smoothed value at each wavelength is taken as the value of the fitted polynomial at the center of the window, which preserves peak shape while reducing high-frequency noise. Spectra were further modelled using both ordinary PLSR and WSPLSR methods.

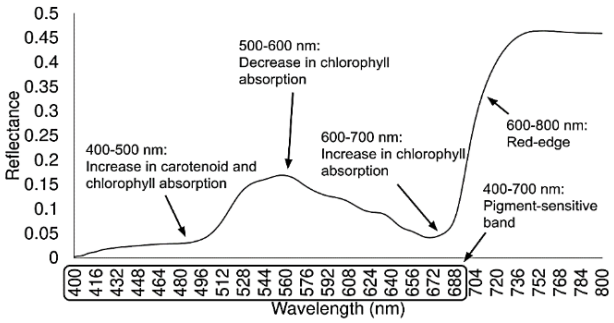

Fig. 1. Near-infrared reflectance spectra of the adaxial surface of a leaf, highlighting characteristic spectral regions.

B. Spectrophotometry

Spectrophotometry is a standard technique for quantitatively assessing the transmitted properties of pigments. It serves as the standard for quantifying concentrations of Chl *a*, Chl *b*, Chl *a*+*b*, and Car in µg/cm², which serves as reference measurements for both regression models. Fresh leaves were harvested and quickly frozen in liquid nitrogen to prevent Chl degradation. Leaves were then finely powdered using a mortar and pestle, ensuring thorough grinding to maximise pigment extraction.

Pigments are extracted with ethanol in a test tube containing the powdered sample. Samples are kept in darkness during extraction to prevent Chl degradation from light exposure, with periodic agitation to enhance extraction efficiency over 24-48 hours at 4°C. Following extraction, centrifugation separates debris, yielding a clear supernatant with extracted pigments. Before spectrophotometric analysis, a blank solution with the same solvent is prepared for calibration. The spectrophotometer is set to measure absorbance at wavelengths 470 nm, 646 nm, and 663 nm.

C. Partial Least Squares Regression

PLSR is a supervised method designed to capture correlations between a set of independent variables, referred to as predictors (X), and a set of dependent variables or responses (Y). This method establishes a linear regression between X and Y by projecting them onto a set of latent variables or components (N). Latent variables are optimised to maximise the covariance between X and Y, expressed as $TP^T + E$ and $UQ^T + F$, respectively, for X and Y. Optimisation is achieved through iterative extraction in each cycle. By computing regression coefficients, PLSR delineates the relationships between the original variables and the dependent variable, facilitating predictions for new observations. T and U are used to project variables from X and Y, while P and Q serve as loading matrices for X and Y. Matrices E and F represent error terms.

D. Wavelength-Selective Partial Least Squares Regression

WSPLSR method is a refined approach derived from the standard PLSR method, aiming to enhance accuracy in spectral analysis. Initially, a PLSR model is trained using all wavelengths, and the regression coefficients for each wavelength are extracted, reflecting their correlation with pigment concentrations obtained via spectrophotometry. Higher absolute values of the regression coefficient indicate stronger associations, crucial for subsequent refinement.

Regression coefficients are then sorted in ascending order, identifying wavelengths with minimal values that might be excluded to streamline the model. Iterative models are sequentially developed, progressively excluding wavelengths with the smallest regression coefficient to optimise correlation. Each iterative model undergoes evaluation through cross-validation, dividing the dataset into 10 folds to ensure reliability. Validation occurs iteratively, where one fold validates while the model trains on the remaining folds, ensuring robustness across subsets.

The evaluation of both ordinary PLSR and WSPLSR models hinges on three key parameters: high R^2 values, low MSE, and a high RPD, all of which are indicative of robust model performance in spectral data analysis. An R^2 value close to 1 indicates a better fit of the model to the data. MSE ranges from a minimum of 0 to an indefinite maximum, reflecting the prediction errors. Values approaching 0 signify the best fit, indicating a closer alignment between predictions and actual values, and reflecting higher accuracy in the model.

III. Results and Discussion

A. Chlorophyll Bands

Chloroplasts are organelles found in plant cells. Inside chloroplasts, there are membranous structures called thylakoids, which are organized into stacks called grana. Thylakoid membranes contain Chl and other pigments that capture light energy during the light-dependent reactions of photosynthesis. Photosynthesis is a process of converting light energy into chemical energy and relies on intricate mechanisms involving specialised protein complexes within chloroplasts. The Chl, located predominantly in the thylakoid membranes, absorbs photons in the blue (400-500 nm) and red (600-700 nm) regions of the electromagnetic spectrum, while reflecting green light (500-600 nm).

Photosystem II (PS II), also known as P680 due to its absorption peak at approximately 680 nm, plays a crucial role in the light-dependent reactions. Protein complex is responsible for the oxidation of water molecules into oxygen, protons, and electrons, facilitated by the oxygen-evolving complex adjacent to P680 in the thylakoid membrane. Excited electrons from P680 are transferred through a series of electron carriers, including plastoquinone, the cytochrome b6f complex, and plastocyanin, which are embedded in the membrane. This process releases energy that pumps protons into the thylakoid lumen, establishing a proton gradient. This gradient drives ATP synthesis via ATP synthase, a process known as photophosphorylation, providing energy for the Calvin cycle. The Calvin Cycle takes place in the stroma of chloroplasts, where it incorporates carbon dioxide from the atmosphere into organic molecules, as illustrated in Fig. 2.

Photosystem II collaborates with Photosystem I (PS I or P700), which absorbs light at 700 nm, to complete the electron transport chain. Electrons from PS II eventually reach PS I, where they are reenergized and utilised in the reduction of NADP+ to NADPH, a process crucial for carbon fixation in the Calvin cycle. Interconnected systems of Photosystem II and Photosystem I orchestrate the capture, transfer, and utilisation of light energy to sustain plant growth through photosynthesis.

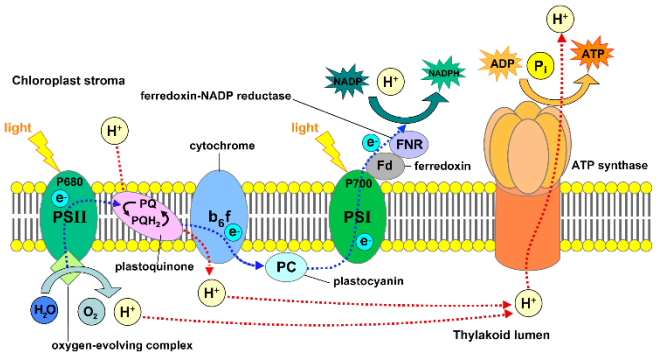

Fig. 2. Light-dependent reactions of photosynthesis in the thylakoid membrane.

B. Carotenoid Band

Chloroplasts contain various Car such as β-carotene, α-carotene, and xanthophylls that absorb light within the 400-500 nm wavelength range. Car harvests light energy and transfers this energy to Chl through singlet–singlet excitation transfer. Singlet–singlet transfer is a lower-energy state transfer used during photosynthesis. Car absorbs excessive energy from Chl through triplet–triplet transfer and releases excessive energy by polyene vibration. Triplet–triplet transfer is a higher-energy state essential for photo-protection. Reactive oxygen species such as singlet oxygen, hydroxyl radicals, and superoxide anion radicals are produced from oxygen and light during photosynthesis. Car with more than eleven conjugated double bonds show a marked capacity to quench singlet oxygen. The mechanism for quenching singlet oxygen is a physical reaction. Car takes up thermal energy from singlet oxygen and releases this energy by polyene vibration.

C. Regression Models

PLSR method showed R^2 values that reveal distinct predictive capabilities among Chl *a*, Chl *b*, and Chl *a*+*b*. Chl a shows superior prediction with an R^2 value of 0.922 compared to Chl *b* (R^2 = 0.853) and Chl *a*+*b* (R^2 = 0.910) across specific spectral bands, as presented in Table 2. Models for Chl *a* and Chl *a*+*b* exceed the RPD threshold of 3, achieving values of 3.571 and 3.34, respectively, indicating effective prediction of Chl concentrations. The Car exhibits a lower R^2 value of 0.787 and a higher value of 2.122 in the 400-500 nm band with the predicted line equation of y = 0.82x + 1.58. PLSR method is effective for predicting Chl *a* and Chl *a*+*b* pigments with predicted line equations of y = 0.943x + 1.48 and y = 0.921x + 2.77, as illustrated in Fig. 3.

TABLE II. ORDINARY PLSR MODEL PARAMETERS.

Pigments	Equations	Components	R^2	MSE	RPD
Chl *a*	y = 0.943x + 1.48	7	0.922	18.967	3.571
Chl *b*	y = 0.866x + 1.15	5	0.853	4.53	2.609
Chl *a*+*b*	y = 0.921x + 2.77	6	0.91	39.629	3.34
Car	y = 0.82x + 1.58	6	0.778	5.375	2.122

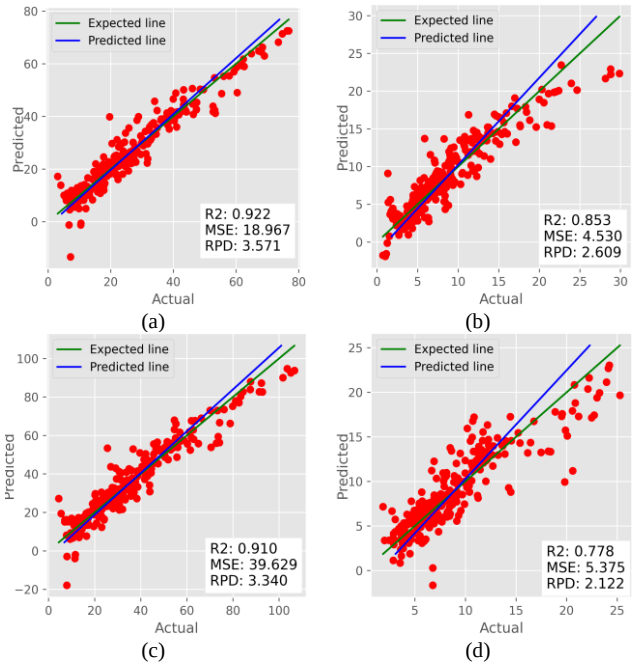

Fig. 3. PLSR models of (a) Chl *a*, (b) Chl *b*, (c) Chl *a*+*b*, and (d) Car. Savitzky–Golay filter was applied with a frame length of 7 and a polynomial order of 2.

Pigment predictability of the WSPLSR method outstands the ordinary PLSR method, especially for Chl *a*, with an R^2 value of 0.978, which outperforms Chl *b* (R^2 = 0.953) and Chl *a*+*b* (R^2 = 0.974). Predicted line equations are y = 0.983x + 0.37, y = 0.976x + 0.12, and y = 0.982x + 0.44, respectively, as presented in Table 3. Car maintains a predictive R^2 value of 0.908 with the predicted line equation y = 0.953x + 0.3. RPD values validate these models: Chl *a* (RPD = 6.761), Chl *b* (RPD = 4.593), Chl *a*+*b* (RPD = 6.2), and Car (RPD = 3.29), highlighting their effectiveness in predicting pigment concentrations in leaves, as illustrated in Fig. 4. Compared with ordinary PLSR, WSPLSR significantly enhanced pigment estimation accuracy by reducing redundant spectral

information and emphasizing informative wavelengths. WSPLSR models achieved higher R^2 values (0.908–0.978), lower MSE, and superior RPD values (3.29–6.76), demonstrating excellent robustness, particularly for chlorophyll pigments. Integration of WSPLSR into NIR spectra offers a promising screening tool for detecting different pigments in leaves, providing farmers with proactive strategies to manage pigment-related stress in agriculture through advanced machine learning regression models.

TABLE III. WSPLSR MODEL PARAMETERS.

Pigments	Equations	Components	Wavelengths	R^2	MSE	RPD
Chl *a*	y = 0.983x + 0.37	10	605	0.978	5.292	6.761
Chl *b*	y = 0.976x + 0.12	13	617	0.953	1.462	4.593
Chl *a+b*	y = 0.982x + 0.44	10	622	0.974	11.503	6.2
Car	y = 0.953x + 0.3	14	641	0.908	2.237	3.29

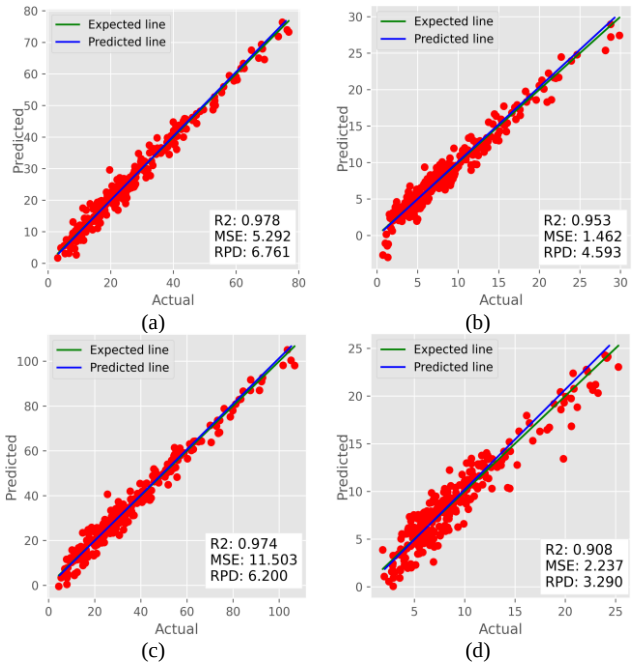

Fig. 4. WSPLSR models of (a) Chl *a*, (b) Chl *b*, (c) Chl *a+b*, and (d) Car. First derivative preprocessing was applied with a frame length of 15 and a polynomial order of 8.

IV. CONCLUSION

WSPLSR significantly enhances the predictive accuracy of NIR spectroscopy over conventional PLSR in estimating various pigments in leaves. While ordinary PLSR exhibits variable predictive performance across different pigments, WSPLSR consistently achieves high R^2 values for all pigments, as further supported by elevated RPD values. This demonstrates the robustness and reliability of WSPLSR as a non-destructive method for accurate pigment estimation, which is crucial for applications in remote sensing platforms such as satellites and unmanned aerial vehicles. By integrating data-driven regression models with NIR spectroscopic analysis, the approach offers valuable insights into precision agriculture, enabling proactive monitoring and management of pigment-related stresses in plants.

Future research could explore the integration of WSPLSR with real-time hyperspectral imaging systems for continuous crop monitoring. Incorporating machine learning algorithms or deep learning frameworks could further enhance the model adaptability to different crop species, environmental conditions, and developmental stages. These advancements can support the development of large-scale, automated systems for early detection of nutrient deficiencies, disease outbreaks, or abiotic stress, ultimately promoting more sustainable and efficient agricultural practices. Extending WSPLSR-based methods to other plant traits beyond pigments—such as moisture content, chlorophyll fluorescence, or structural parameters—could broaden its applicability in innovative farming technologies.

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