



E-ISSN: 2707-8418

P-ISSN: 2707-840X

[Journal Website](#)

IJSSE 2026; 7(1): 49-55

Received: 05-02-2026

Accepted: 07-03-2026

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Optimizing ground sampling distance for rice leaf chlorophyll estimation using UAV multispectral imagery and vegetation indices

Le Van CanhDOI: <https://www.doi.org/10.22271/2707840X.2026.v7.i1a.63>

Abstract

This study aimed to determine the optimal Ground Sampling Distance (GSD) of UAV multispectral imagery for estimating Leaf Chlorophyll Content (LCC) in rice using vegetation indices (VIs). The experiment was conducted on the J02 rice variety in Phu Tho Province during the heading stage. Multispectral UAV imagery was acquired at three GSD levels, including 2 cm, 4 cm, and 6 cm, corresponding to one, two, and three times the width of mature rice leaves, respectively. Simultaneously, LCC was directly measured using a SPAD meter at three sampling points within each field plot to ensure the representativeness of field data. Vegetation indices extracted from the UAV multispectral imagery included CIg (Chlorophyll Index Green), GNDVI (Green Normalized Difference Vegetation Index), NDRE (Normalized Difference Red Edge Index), and NDVI (Normalized Difference Vegetation Index). The correlation analysis results indicated that all vegetation indices achieved the highest correlation with LCC at GSD = 4 cm. During the rice heading stage, the CIg index showed the strongest correlation with chlorophyll content, followed by NDRE, while NDVI and GNDVI exhibited lower performance. The study confirms that GSD significantly affects the accuracy of LCC estimation from UAV multispectral imagery, and that selecting a GSD equivalent to twice the width of rice leaves is optimal. Furthermore, during the heading stage, the CIg and NDRE indices are recommended for evaluating rice LCC instead of NDVI.

Keywords: GSD, multispectral UAV, leaf chlorophyll content, rice plant

Introduction

Leaf chlorophyll content (LCC) is one of the most important physiological indicators in crops, playing a direct role in the photosynthetic process of rice plants and other vegetation. LCC reflects the capacity of plants to absorb and convert light energy and serves as a valuable indicator for assessing plant growth, development, and overall health status ^[12]. Monitoring and evaluating LCC can help elucidate the relationship between plant physiological characteristics and environmental conditions, thereby supporting the assessment of crop growth performance, stress status, and potential yield outcomes ^[13,19].

The spectral reflectance characteristics of chlorophyll provide an important basis for estimating its content using UAV-based multispectral imagery. Numerous studies have demonstrated that the green and red spectral bands are highly effective for detecting and estimating chlorophyll content ^[4], while the near-infrared (NIR) band has also been shown to be useful for assessing the physiological status of crops ^[4,18]. In addition, the red-edge spectral region is considered one of the most sensitive indicators of chlorophyll variation and has been widely applied in studies related to biomass estimation, crop growth monitoring, and vegetation health assessment ^[8,25].

In recent years, unmanned aerial vehicles (UAVs) equipped with multispectral sensors have become an effective tool for crop monitoring due to their ability to acquire data rapidly, provide high spatial resolution, and offer flexible survey scheduling. UAV-based multispectral sensors can capture imagery within the visible spectrum, including the blue, green, and red bands, while also incorporating spectral bands that are highly sensitive to vegetation dynamics, such as the red-edge and near-infrared (NIR) regions ^[21].

Numerous studies have demonstrated that vegetation indices derived from UAV multispectral imagery, including the Normalized Difference Vegetation Index (NDVI), Green Normalized Difference Vegetation Index (GNDVI), Normalized Difference Red Edge Index (NDRE), and Chlorophyll Index Green (CIg), are effective predictors of leaf

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chlorophyll content (LCC) in rice crops. These indices exhibit strong correlations with Chl because they are based on vegetation reflectance characteristics in the green, red, red-edge, and near-infrared spectral bands [1, 3, 22]. Among these indices, NDVI is widely used to evaluate crop growth status and vegetation development; GNDVI has shown high sensitivity to variations in chlorophyll content; NDRE performs particularly well in estimating chlorophyll content during later growth stages due to its use of the red-edge spectral region; and CIg has been reported to exhibit a strong correlation with canopy chlorophyll content [1, 15].

However, the accuracy of chlorophyll content estimation from UAV imagery depends not only on the spectral characteristics of the sensor but is also significantly influenced by the spatial resolution of the imagery, commonly expressed as Ground Sampling Distance (GSD). GSD determines the level of spatial detail represented in an image; smaller GSD values provide greater capability for capturing canopy structure and distinguishing target objects. Conversely, as GSD increases, spectral mixing among crop canopies, soil backgrounds, and shadows may occur, thereby reducing the accuracy of vegetation indices and chlorophyll estimation models. Furthermore, variations in GSD can directly affect spectral reflectance values and the stability of vegetation indices derived from multispectral imagery.

Although numerous studies have applied UAV-based multispectral imagery for chlorophyll content estimation,

investigations into the effects of GSD on the accuracy of LCC estimation remain limited, particularly for rice cultivation under Vietnamese agricultural conditions. Determining an appropriate GSD is important not only for improving the accuracy of LCC estimation models but also for optimizing flight altitude, data acquisition efficiency, and UAV operational costs in practical agricultural applications. Therefore, examining the influence of GSD on the estimation of chlorophyll content from UAV-based multispectral imagery is both scientifically valuable and practically relevant for advancing precision agriculture applications.

Materials and Methods

Experimental Site Design

The experimental study was conducted in Phung Nguyen Commune, Phu Tho Province, Vietnam, a traditional rice-growing area with favorable soil conditions, well-developed irrigation infrastructure, and convenient transportation networks that support rice cultivation. More importantly, the study area is characterized by relatively flat terrain and the absence of tall surrounding structures, providing suitable conditions for UAV flight operations and image acquisition. The experimental field was divided into plots measuring 10 m × 10 m, with a 1 m buffer zone between adjacent plots. A total of 12 experimental plots were established for this study (Figure 1).

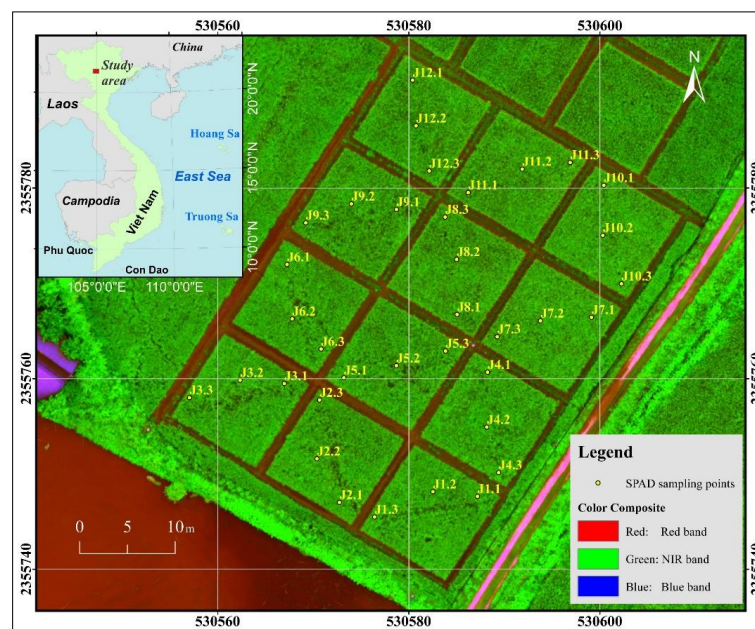


Fig 1: Study Area

The Japonica rice variety J02, which occupies a large proportion of the cultivated area in the study region, was selected for the experiment. This high-yielding cultivar originates from Japan and is widely grown under local farming conditions. J02 is characterized by an erect and compact leaf architecture. At the mature stage, the average leaf width is approximately 2.06 cm [6]. This morphological characteristic provides an important basis for selecting appropriate GSD levels to ensure accurate identification of rice leaves in UAV-based multispectral imagery. Previous studies on chlorophyll content estimation in rice using UAV multispectral imagery have employed various GSD levels. For example, Ban *et al.* (2022) conducted their study using

images with GSD of approximately 6cm [1], Wang *et al.* (2023) used a GSD of approximately 5.4 cm, [22], and Pham *et al.* (2025) performed image acquisition with a GSD of 4 cm [16]. These studies indicate that GSD values below 6 cm are generally sufficient for extracting spectral information required for chlorophyll estimation models. Based on the leaf dimensions of the J02 variety and findings from previous studies, this research selected three GSD levels for evaluation: 2 cm, 4 cm, and 6 cm. These GSD values correspond approximately to one, two, and three times the average width of mature rice leaves, respectively. Data were collected during the heading stage, when rice plants reach maximum canopy development. A baseline GSD

of 2 cm was selected because it allows the identification of pixels located entirely within rice leaves, thereby minimizing spectral mixing between leaf surfaces and surrounding objects. This approach improves the accuracy of spectral information extraction for LCC estimation.

To evaluate the effects of GSD on LCC estimation, chlorophyll measurements were collected from three evenly distributed sampling locations within each experimental plot (Figure 1). A total of 36 sampling points were established across the study area. The coordinates of all sampling points were measured using real-time kinematic GNSS technology to facilitate accurate co-registration and extraction of corresponding spectral information from the UAV imagery.

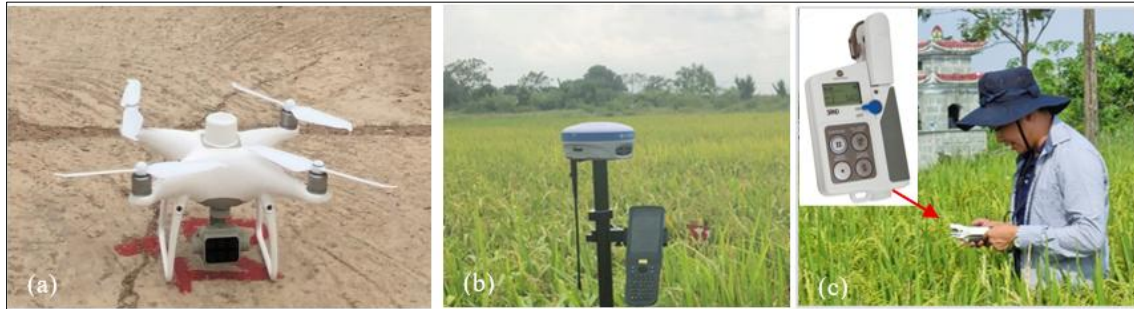


Fig 2: Field Data Collection Equipment

a. DJI Phantom4 Multispectral, b. GNSS Stonex 900A, c. Chlorophyll measurement using the LCPM-A10 chlorophyll meter.

The locations of the leaf chlorophyll sampling points were determined using a Stonex S900A dual-frequency GNSS receiver (Figure 2b) operating in real-time kinematic (RTK) mode. Leaf chlorophyll content (LCC) at the ground sampling points was measured using a handheld Chlorophyll Meter LCPM-A10 (Figure 2c). This instrument is widely used for chlorophyll assessment and is capable of measuring leaf chlorophyll content over a minimum sampling area of 2×2 mm, with an accuracy of ± 3 SPAD units and a measurement time of less than three seconds [14].

Multispectral UAV data were acquired under clear-sky conditions with stable solar illumination. Image acquisition flights were conducted between 11:00 and 12:00 local time to minimize the effects of illumination variability. Flight altitudes corresponding to GSD values of 2 cm, 4 cm, and 6 cm were 38 m, 76 m, and 114 m, respectively. Both forward and side image overlap were set to 75%.

Field measurements of leaf chlorophyll content were conducted immediately after the completion of UAV image

Equipment and Data Collection

A DJI Phantom 4 Multispectral RTK (P4M) unmanned aerial vehicle (UAV) equipped with a multispectral imaging system was used to acquire multispectral imagery in this study (Figure 2a). The P4M is equipped with five multispectral sensors, including red (650 nm), blue (450 nm), green (560 nm), and red-edge (730 nm) bands, each with a spectral bandwidth of ± 16 nm, as well as a near-infrared (NIR) sensor centered at 840 nm with a spectral bandwidth of ± 26 nm. In addition, the platform is equipped with a conventional RGB camera for acquiring true-color imagery [5]. Consequently, each image acquisition simultaneously generates six images corresponding to six spectral channels, including five multispectral bands and one RGB image.

acquisition. At each sampling location, previously georeferenced using GNSS/RTK, chlorophyll measurements were taken from three rice leaves, and the average value was used as the representative LCC measurement for that sampling point.

Image Processing and Vegetation Indices Extraction

The P4M UAV acquires imagery from six channels; however, the RGB imagery was not used in this study. The five multispectral bands, including red, blue, green, red-edge, and near-infrared (NIR), were radiometrically calibrated using data recorded by the onboard sunlight sensor for each image during acquisition. This calibration process was performed prior to image processing to ensure the consistency and reliability of spectral reflectance values.

The multispectral UAV imagery was processed using Agisoft Metashape Professional software to generate multispectral orthomosaics corresponding to the five spectral bands used in this study. Vegetation indices, including the Cig, NDRE, NDVI and GNDVI, were derived from the multispectral orthomosaics using the equations presented in Table 1.

Table 1. Vegetation index Equations

Vegetation Indices	Equation	References
Chlorophyll Index Green (Cig)	$Cig = (R_{NIR}/R_G) - 1$	[11]
Green Normalized Difference Vegetation Index (GNDVI)	$GNDVI = (R_{NIR} - R_G)/(R_{NIR} + R_G)$	[2]
Normalized Difference Red Edge Index (NDRE)	$NDRE = (R_{NIR} - R_{RE})/(R_{NIR} + R_{RE})$	[9]
Normalized Difference Vegetation Index (NDVI)	$NDVI = (R_{NIR} - R_R)/(R_{NIR} + R_R)$	[17]

Where R_{NIR} , R_{RE} , R_R , and R_G represent the spectral reflectance values obtained from the near-infrared, red-edge, red, and green bands of the multispectral imagery, respectively.

Correlation Analysis

The Pearson correlation coefficient (r) was used to quantify the strength of the relationship between variables. Variations in spectral reflectance are associated with differences in vegetation characteristics, which in turn influence the degree of correlation between variables and affect data variability

[21]. Therefore, the Pearson correlation coefficient was selected as the primary indicator for evaluating the influence of GSD on LCC estimation. The value of r was calculated using Equation (1).

$$r = \frac{\sum_1^n (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_1^n (x_i - \bar{x})^2} \cdot \sqrt{\sum_1^n (y_i - \bar{y})^2}} \quad (1)$$

Where x_i and y_i are the observed values of variables X and Y , respectively; $\bar{x}$ and $\bar{y}$ are the mean values of X and Y , respectively; and n is the number of observations.

The magnitude of Pearson's correlation coefficient (r) was used to classify the strength of the relationship between vegetation indices and leaf chlorophyll content (LCC), as shown in Table 2.

Table 2: Correlation Strength [7]

Correlation direction	Correlation Strength	Correlation Coefficient (r)
Positive correlation	High	$0.50 \leq r \leq 1.0$
	Moderate	$0.30 \leq r < 0.50$
	Low	$0.10 \leq r < 0.30$
No correlation		0.0
Negative correlation	Low	$-0.30 < r \leq -0.10$
	Moderate	$-0.50 < r \leq -0.30$
	High	$-1.0 \leq r \leq -0.50$

Leaf Chlorophyll Content Estimation and Accuracy Assessment

After performing the correlation analysis between leaf chlorophyll content (LCC) and vegetation indices (VIs), the vegetation index exhibiting the strongest correlation with LCC was selected for LCC estimation across the entire study area. A linear regression model was adopted to estimate LCC from the selected vegetation index using UAV multispectral imagery at GSD levels of 2 cm, 4 cm, and 6 cm, as expressed in Equation (2).

$$Y = aX + b \quad (2)$$

Where Y is the estimated leaf chlorophyll content (LCC), X is the selected vegetation index, and a and b are regression coefficients.

The accuracy of the LCC estimation models was evaluated using the coefficient of determination (R^2) and the root mean square error (RMSE). Both metrics were calculated based on the differences between the measured LCC values at the sampling points and the corresponding values estimated by the regression model, according to Equations (3) and (4).

$$R^2 = 1 - \frac{\sum_{i=1}^n (L_i - X_i)^2}{\sum_{i=1}^n (L_i - \bar{L})^2} \quad (3)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^n (L_i - X_i)^2}{n}} \quad (4)$$

Where: L_i is the measured leaf chlorophyll content (LCC) at sample point i ; X_i is the corresponding estimated LCC value obtained from the regression model; $\bar{L}$ is the mean measured LCC of all samples; and n is the total number of samples.

Results and Discussion

Correlation Analysis Results

The correlation analysis between SPAD values and vegetation indices derived from UAV multispectral imagery showed that all investigated vegetation indices exhibited positive correlations with leaf chlorophyll status in rice plants (Figure 3). The Pearson correlation coefficients ranged from 0.701 to 0.758, indicating that NDVI, NDRE, GNDVI, and CIg were all capable of capturing variations in chlorophyll

content. These findings are consistent with previous studies reporting strong relationships between UAV-derived vegetation indices and chlorophyll content or SPAD measurements in rice crops [1, 3, 22].

The summarized results presented in Figure 3p indicate that GSD = 4 cm consistently produced the highest correlation coefficients for all evaluated vegetation indices. Specifically, the correlation coefficients reached 0.725 for NDVI, 0.754 for NDRE, 0.721 for GNDVI, and 0.758 for CIg. These results suggest that a GSD of 4 cm provides the most suitable spatial resolution for extracting spectral information related to chlorophyll content under the conditions of this study. Similar findings have been reported by Wang *et al.* [22] and Ban *et al.* [1], who achieved high chlorophyll estimation accuracy using UAV multispectral imagery with GSD values ranging from approximately 4 to 6 cm.

Among all investigated vegetation indices, CIg consistently exhibited the strongest relationship with chlorophyll content across all GSD levels. As shown in Figure 3k, Figure 3m, Figure 3n, and particularly in the summary presented in Figure 3p, the correlation coefficients between CIg and SPAD values reached 0.733, 0.758, and 0.740 for GSD values of 2 cm, 4 cm, and 6 cm, respectively. The superior performance of CIg can be attributed to its sensitivity to variations in chlorophyll concentration. This index combines information from the green and near-infrared spectral bands, which are closely associated with chlorophyll absorption and vegetation reflectance characteristics [10]. Consequently, CIg was selected for subsequent LCC estimation and accuracy assessment.

NDRE also demonstrated strong and stable correlations across all spatial resolutions, reaching its highest value ($r = 0.754$) at GSD = 4 cm (Figure 3e). As illustrated in Figure 3p, NDRE maintained correlation coefficients greater than 0.71 at all investigated GSD levels. This result agrees with previous studies indicating that the red-edge spectral region is particularly effective for monitoring plant physiological status and chlorophyll content during advanced growth stages [3, 22].

In contrast, NDVI and GNDVI showed comparatively weaker relationships with chlorophyll content, with correlation coefficients ranging from 0.701 to 0.725 (Figures 3a-c and Figures 3g-i). Although both indices were positively correlated with SPAD values, their performance was lower than that of NDRE and CIg. One possible explanation is that

NDVI tends to experience spectral saturation under dense canopy conditions, such as those observed during the rice heading stage [20]. Similarly, although GNDVI is generally sensitive to chlorophyll variation, its performance may still be influenced by canopy structure and illumination conditions during image acquisition [23].

The highest spatial resolution (GSD = 2 cm) did not produce the strongest correlations, despite providing the greatest level of spatial detail. As shown in Figure 3a, Figure 3d, Figure 3g, and Figure 3k, the correlation coefficients obtained at GSD = 2 cm were consistently lower than those observed at GSD = 4 cm. This finding suggests that increasing spatial resolution

beyond a certain threshold does not necessarily improve chlorophyll estimation performance. Extremely high spatial resolution may increase local spectral variability caused by leaf shadows, within-canopy reflectance heterogeneity, geometric misalignment among spectral bands, and other sources of noise. Moreover, the larger number of pixels associated with finer spatial resolution can amplify spectral variability among leaves and within individual canopy components. Similar observations were reported by Zhang *et al.* [24], who found that excessively high spatial resolution may increase spectral uncertainty due to canopy structural effects and non-uniform illumination conditions.

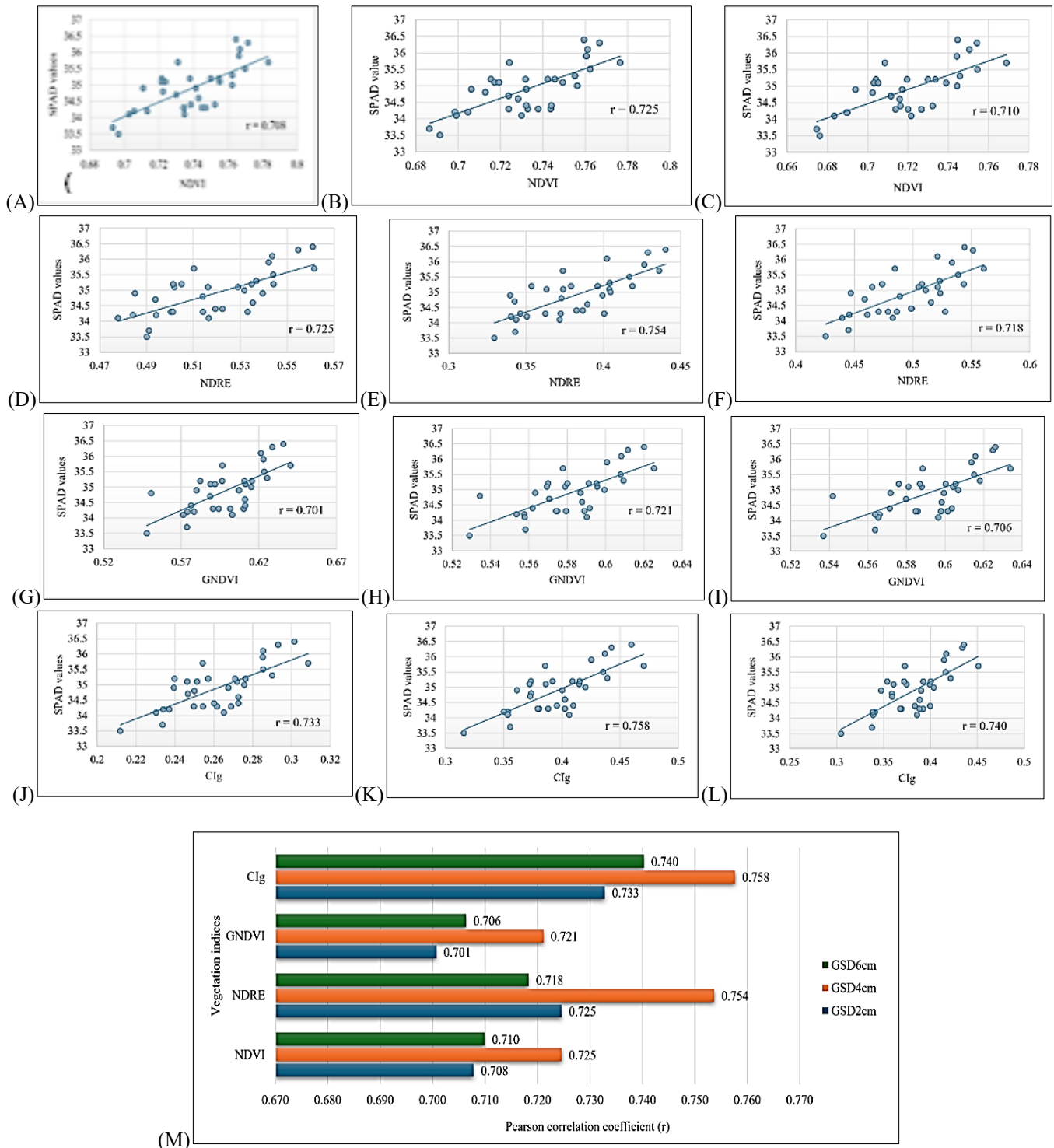


Fig 3: Correlations between SPAD values and vegetation indices (VIs) derived from UAV multispectral imagery at GSDs of 2 cm, 4 cm, and 6 cm: NDVI (a-c), NDRE (d-f), GNDVI (g-i), Clg (k-n) and summary of Pearson correlation coefficients (p)

In contrast, at a GSD of 6 cm, the correlation coefficients tended to decrease for most vegetation indices, as evident in Figure 3c, Figure 3f, Figure 3i, and Figure 3n. This reduction may be attributed to the mixed-pixel effect, whereby a single image pixel contains spectral information from multiple surface components, including rice leaves, standing water, soil background, and shadows. Such spectral mixing reduces the ability of vegetation indices to accurately capture chlorophyll-related spectral characteristics. These findings are consistent with those reported by Xue and Su ^[23], who suggested that increasing pixel size may reduce the sensitivity of vegetation indices to variations in leaf chlorophyll content due to the influence of background effects and canopy structure.

3.2 Leaf Chlorophyll Content Estimation

Among the evaluated vegetation indices, CIg consistently exhibited the strongest correlation with LCC across all investigated GSD levels. Therefore, CIg was selected to develop regression models for LCC estimation under GSD values of 2 cm, 4 cm, and 6 cm. The performance of the resulting models was assessed using the coefficient of determination (R^2) and root mean square error (RMSE) (Table 3).

Table 3: Performance of CIg-Based Regression Models for LCC Estimation Under Different GSD Levels

GSD	Regression equation	R^2	RMSE
2cm	$y = 23.839x + 28.653$	0.537	2.22
4cm	$y = 15.968x + 28.571$	0.575	1.96
6cm	$y = 16.221x + 28.703$	0.548	2.34

The results show that the regression models achieved moderate to strong predictive performance, with R^2 values ranging from 0.537 to 0.575 and RMSE values ranging from 1.96 to 2.34. Among the three tested spatial resolutions, the model developed using GSD = 4 cm produced the best performance, achieving the highest coefficient of determination ($R^2 = 0.575$) and the lowest prediction error (RMSE = 1.96). In comparison, the models based on GSD = 2 cm and GSD = 6 cm yielded lower R^2 values of 0.537 and 0.548, respectively, with corresponding RMSE values of 2.22 and 2.34.

The results suggest that increasing spatial resolution does not necessarily improve LCC estimation accuracy. Instead, an intermediate GSD may provide more reliable spectral information for chlorophyll assessment. For the J02 rice variety investigated in this study, a GSD of approximately 4cm appears to represent a suitable between spatial detail and spectral consistency, resulting in the most accurate CIg-based LCC estimation.

Conclusion

Ground Sampling Distance (GSD) significantly influences the accuracy of leaf chlorophyll content (LCC) estimation from UAV-based multispectral imagery. Under the conditions of this study, a GSD of 4 cm provided the best estimation performance, achieving an effective balance between preserving spectral information, reducing image noise, and minimizing spectral mixing effects. These findings may serve as a useful reference for selecting appropriate flight altitudes in UAV-based studies of rice growth and nutrient monitoring.

At the heading stage, when the canopy of the J02 rice variety was fully developed, the CIg and NDRE indices exhibited stronger correlations with LCC than the widely used NDVI. This result suggests that NDVI tends to experience spectral saturation under dense canopy conditions, thereby reducing its sensitivity to variations in leaf chlorophyll content.

The selection of GSD based on the morphological characteristics of the target crop proved effective in optimizing the extraction of spectral information from UAV multispectral imagery. The results suggest that, for rice crops, a GSD approximately twice the width of a mature leaf may represent an appropriate threshold for balancing spatial detail and spectral signal stability. This finding provides valuable guidance for optimizing flight planning and improving the effectiveness of UAV-based crop growth monitoring applications.

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