Applying Spectral Fractal Dimension to Predict the SPAD Value of Rice Leaves Under Disease Stress

YiFei Cao

Nanjing Agricultural University

Huanliang Xu

Nanjing Agricultural University

Jin Song

Nanjing Agricultural University

Yao Yang

Nanjing Agricultural University

Xiaohui Hu

Jiangxi Vocational College of Mechanical & Electrical Technology

Korohou Tchalla Wiyao

Nanjing Agricultural University

Zhaoyu Zhai (✉ zhaoyu.zhai@njau.edu.cn)

Nanjing Agricultural University <https://orcid.org/0000-0003-2023-4894>

Research

Keywords: Hyperspectral, fractal dimension, SPAD value, vegetation index, disease stress, rice

Posted Date: December 8th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1084650/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Applying spectral fractal dimension to predict the SPAD value of rice leaves under disease stress

YiFei Cao ¹, Huanliang Xu ², Jin Song ¹, Yao Yang ², Xiaohui Hu ³, Korohou Tchalla Wiyao ¹, Zhaoyu Zhai ^{2,*}

*Correspondence: Zhaoyu Zhai (zhaoyu.zhai@njau.edu.cn)

Abstract

Background: The chlorophyll content is a vital indicator for reflecting the photosynthesis ability of plants and it plays a significant role in monitoring the general health of plants. Since the chlorophyll content and the soil-plant analysis development (SPAD) value are positively correlated, it is feasible to estimate the SPAD value by calculating the vegetation indices (VIs) through hyperspectral images, thereby estimating the chlorophyll content. However, current indices simply adopted few wavelengths of the hyperspectral information, which may decrease the estimation accuracy. Besides, few researches explored the applicability of VIs over plant leaves under disease stress.

Methods: In this study, the SPAD value was estimated by calculating the fractal dimension of hyperspectral curves, ranging from 420 to 950 nm. The correlation between the SPAD value and wavelengths under disease stress was analyzed. In addition, a SPAD prediction model was built upon the combination of selected indices and 4 machine learning methods, including decision tree (DT), partial least square regression (PLSR), support vector regression (SVR), and back propagation neural network (BPNN). The performance of these models was compared through the correlation of determination, root mean square error, and relative error.

Results: The results suggested that the SPAD value of rice leaves under different disease levels were sensitive to different wavelengths, meaning that the fixed wavelength selection in current indices may achieve poor estimation results. Compared with current VIs, a stronger positive correlation was detected between the SPAD value and our proposal, reaching an average correlation coefficient of 0.8263. For the prediction model, the one built with our proposal and SVR achieved the best performance, reaching R^2 , RMSE, and RE at 0.8752, 3.7715, and 7.8614%, respectively.

Conclusions: This work provides an in-depth insight for accurately and robustly estimating the SPAD value of rice leaves under disease stress, and our proposal is of great significance for monitoring the chlorophyll content in large-scale fields non-destructively.

Keywords: Hyperspectral; fractal dimension; SPAD value; vegetation index; disease stress; rice

Background

During the photosynthesis process, chlorophyll, as a plant pigment, is an important material for absorbing light energy [1]. The chlorophyll content is one of the essential factors that directly affects plant growth, and therefore it can be used to reflect stress of plants [2-4]. For instance, when a disease is spreading among plants, their leaves usually turn from green to yellow, eventually even to brown and white. This change means that the chlorophyll content in plants is decreased, leading to the fact that plants may have difficulty in photosynthesis [5, 6]. Previous studies have demonstrated that the chlorophyll content of infected plant leaves is negatively correlated with the severity of plant diseases [7]. As a result, timely and accurately evaluating the chlorophyll content of plant leaves is an efficient measure for monitoring plant diseases.

At current, the soil-plant analysis development (SPAD) meter [8] is widely adopted to evaluate the chlorophyll content due to its advantages like low-cost, ease of use, nondestructive testing, etc. Various publications have reported that SPAD value and the chlorophyll content are positively correlated [9, 10]. Therefore, it is possible to directly measure the SPAD value for monitoring the general health of plants, instead of conducting chlorophyll measurements through laboratory tests. However, hand-held SPAD instruments are not suitable for measurements in large-scale fields [11].

For overcoming this scalability issue, researchers are now applying hyperspectral sensor to the agricultural domain [12, 13], thanks to the rapid development of the remote sensing technique. For evaluating the chlorophyll content, measuring the SPAD value by hyperspectral images has drawn worldwide attention and achieved great success [14-17]. Zhang et al. [18] combined multiple wavelength regions to define a novel index for evaluating the relative chlorophyll content in sugar beet. The performance of this index is verified through a three-year field experiment, achieving the best prediction accuracy of SPAD value with the coefficient of determination (R^2) of 0.83. Yu et al. [19] predicted the SPAD value by considering the chlorophyll sensitive bands and the range of water absorption. The random forest algorithm is used to analyze the correlation between spectral indices and SPAD value. According to the experimental result, the correlation coefficient reached above 0.75 in the range of the chlorophyll sensitive band (600-690 nm), red edge region (720-760 nm), and water absorption band (1400-1490, 1900-1980 nm). Shu et al. [9] detected that the exposure to different backgrounds in the hyperspectral images

may negatively affect the plant chlorophyll diagnosis. After removing the background, the correlation coefficient between selected indices and the SPAD value is significantly improved, reaching at 0.75.

Judging from above, various VIs have been adopted to evaluate the chlorophyll content of plants. Although these VIs could achieve promising results, most of them are usually calculated based on the combination of several visible spectrum like red, green, and blue edge regions, which means that some wavelength regions are not considered. The ignorance of certain regions may result in the loss of spectral information related to the chlorophyll content evaluation, thereby decreasing the estimation accuracy [20, 21]. Meanwhile, few investigations contributed to the evaluation of the chlorophyll content for plants under disease stress. As the disease condition becomes worse, the chloroplast and cell structure in plant leaves would be damaged. When the chlorophyll content is reduced, the SPAD value would decrease as well, and even exceed the estimated range of current VIs [6, 22]. It is also worth noting that the reflectance of visible spectrum would become higher when plants are infected by diseases. Furthermore, changes of spectrum caused by geometric configuration, shape, and reflectance would also have an influence on the accuracy of the SPAD value evaluation [23, 24]. Conclusively, current VIs cannot fully detect changes of the SPAD value in diseased plant leaves [25]. There is an urgent need to develop new evaluation methods by considering more spectral information for overcoming the accuracy issue.

As a popular branch in the mathematics domain, fractal dimension is potentially applicable to analyze the spectral information [26]. A fractal dimension can be seen as a statistical index that characterizes patterns by quantifying the complexity as a ratio of the change in detail to the change in scale. The major benefit of fractal dimension is its sensitivity to changes in spectral information [27], caused by geometric configuration, shape, reflectance, etc. These changes are the main factors that reflect the general health of plants [28, 29]. By calculating the fractal dimension over the spectrum of diseased leaves, adequate spectral information could be obtained. Under this circumstance, the evaluation of the SPAD value depends on the use of all spectral bands, instead of selected sensitive ones, which may improve the evaluation accuracy [30]. Moreover, the fractal dimension is also able to quantify the complexity the spectrum due to its advantageous nature [27, 31, 32].

In this work, we apply the fractal dimension to analyze the hyperspectral information for evaluating the SPAD value of diseased plants. This proposal can be treated as a novel vegetation index, namely spectral fractal dimension index (SFDI). After comparing several machine learning models, including

support vector regression (SVR), decision tree (DT), partial least squares regression (PLSR), and back propagation neural network (BPNN), we select the optimal one and combine SFDI with it to establish a SPAD value evaluation model. Results from this work demonstrate that compared with current VIs like MSAVI, NDVI, PRI, and so on, SFDI can accurately evaluate the SPAD value in plants under different disease levels, thereby estimating the chlorophyll content accordingly.

Materials and methods

Experimental design

A moderately resistant rice cultivar, namely ‘Nanjing 9108’, is selected as the experimental material. After soaking and germinating, we planted the seeds in a facility environment with an average temperature of 30°C and air humidity of 75%. For lowering the planting density, emerged rice are transplanted to individual pots and each pot had 3 plants. In total, 50 plants in good health are finally chosen to be infected by the *Xanthomonas Campestris* (shown in Figure 1). When the 5th leaf of rice started emerging, we used a pair of scissors with dipped pathogenic bacteria to cut off the top leaf of healthy rice for the purpose of infection, as presented in Figure 2.

Fig. 1 Cultivation of the pathogenic bacteria

Fig.2 Infection of the bacterial blight

For obtaining the hyperspectral information, we adopted a push broom hyperspectral imaging system (*Isuzu Optics Corp, Taiwan, China.*). The main components of this imaging system and their parameters are listed in Table 1.

Tab. 1 Main components of the imaging system and parameters

Parameters	Value (unit)
Spectral camera	Raptor EM285
Spectral range	373~1033 nm
Dispersion	97.5nm/mm
Spectral resolution	2.8 nm

Image size	6.15mm*14.2mm
Spatial resolution	Spot diameter <9 um
Aberration	Halo <1.5 um, trapezoid <1 um
Aperture	F/2.4.
Light input	21V/200W halogen light
Efficient	50%

The system software is composed of Spectral-image and HSI Analyzer. Totally, 306 bands, ranging from 373-1033 nm, are selected for obtaining the hyperspectral information of rice leaves.

In general, the workflow of predicting the SPAD value of diseased rice leaves is illustrated in Figure 3. First, we preformed pre-processing operations on the collected raw hyperspectral, including HSI correction and smoothing. Then, SFDI and selected VIs are calculated. We also used the SPAD meter to measure the SPAD value. The correlation between indices and SPAD value is analyzed. Lastly, performance of machine learning based SPAD value prediction models are assessed.

Fig. 3. Workflow of predicting the SPAD value of diseased rice leaves

Hyperspectral image acquisition and pre-processing

Hyperspectral image acquisition

Before imaging, the light source is turned on and preheated for 5 minutes to produce a stable light source. The parameters of imaging for collecting hyperspectral in this experiment are presented in Table 2.

Tab. 2 Parameters of imaging in this experiment

Parameters	Values
Lens focal length	35 mm
Object distance	27 cm
Light source	130 lx
Exposure time	10 ms

In order to eliminate background noise, leaves are fixed upon a black cardboard before imaging. Next, the samples, along with the black cardboard, are placed on a shifting platform. The duration of measurement last for 3 weeks. We measured two leaves of each sample rice every other day and the HSI of selected leaves are collected for 10 times. Finally, we obtained 500 hyperspectral images in total. The imaging system is shown in Figure 4.

Fig. 4 Schematic diagram of the hyperspectral imaging system

Considering the moving speed of the shifting platform and the exposure duration may have an influence on the resolution of images and cause distortion, we calibrate the obtained images by following the Equation (1) to reduce noise [33], and therefore, to improve the stability and accuracy of further analysis.

$$R(i) = \frac{I_r(i) - I_d(i)}{I_w(i) - I_d(i)} \quad (1)$$

Where $R(i)$ denotes the reflectance, $I_r(i)$ denotes the uncalibrated reflectance of the obtained images, $I_w(i)$ denotes the reflectance of a white panel, and $I_d(i)$ denotes the substitute for dark current and noise when the camera shutter is closed.

Disease level determination

After segmenting the obtained 500 original images, we build a dataset with 1000 hyperspectral images and each image only contains a single leaf. According to the GB/T 17980.19-2000 standard, the rice bacterial blight disease level can be divided into 6 categories, listed in Table 3.

Tab. 3 Classification standard of bacterial blight

Disease level	Symptoms
---------------	----------

Level 0	No clear spot is shown.
Level 1	It appears 2-3 cm white spots, or even few brown spots are shown. The spot area is account for 10% of the leaf.
Level 2	The length of appeared spots is less than a quarter of the leaf's length, or the spot area is account for 20% of the leaf.
Level 3	The length of appeared spots is between a quarter and half of the leaf's length, or the spot area is account for 20-49% of the leaf.
Level 4	The length of appeared spots is between a half and three quarters of the leaf's length, or the spot area is account for 50-74% of the leaf.
Level 5	The length of appeared spots reaches beyond three quarters of the leaf's length, or the spot area is account for more than 75% of the leaf.

131 In the dataset, the number of rice leaf images labelled by level 0 to 5 is 200, 170, 160, 200, 140, and
132 130, respectively. The leaf images with different labels are displayed in Figure 5.

Fig. 5 Leaves under different disease levels

133 **Hyperspectral information pre-processing**

134 For reducing the noise caused by the imaging system and environment, hyperspectral images are
135 synthesized by the HSI Analyzer. After extracting a 50×50 region of interest (ROI) through ENVI 5.1x
136 (Research System Inc., boulder, CO., USA), we smooth the original spectrum and calculate the average
137 spectrum.

138 Smoothing is widely used to eliminate the interference of high-frequency noise in raw spectral data
139 and to improve the spectral signal-to-noise ratio. Savitzky-Golay (SG) algorithm is a popular method to
140 smooth the raw spectrum by calculating the average value of a set of sample raw spectrum following a
141 moving smoothing window [34]. Zhou et al.[35] used SG smoothing methods to smooth the raw
142 hyperspectral image and highlight the differences of different maize seeds, and established a model to
143 identify different corn varieties. Huang et al [36] utilized the SG smoothing to reduce the noise of raw
144 spectral data, and established a promising method, SG-PCA-RF model, for recognizing *M. micrantha* in

the wild. Since various researches have reported its effectiveness, we adopt SG algorithm in this work as well. The SG smoothing filter has a kernel size of $5 \times 5 \times 5$ and the polynomial order is 3. The filter calculates the filtered value at the central node of the kernel. The smoothing method based on the SG convolution uses the least squares fitting coefficient as the digital filter response function to perform convolutional smoothing. Figure 6 and 7 shows the original spectrum and the spectrum after smoothing.

Fig. 6 Original reflectance curve

Fig. 7 Reflectance curve after SG smoothing

It is noted that both ends of the hyperspectral information contain more noise. Taking into account the number of subsequent data labeling and model computations, Pi et al [37] removed the less informative and noisy 40 bands at both ends of hyperspectral for data pre-processing. Meanwhile, due to the light and other factors during HSI acquisition, the noise at both ends is high, leading to increasing complexity during computation. Therefore, we removed both ends and the wavelength ranging from 420 to 950 nm is maintained for further analysis.

SPAD value acquisition

Many studies have demonstrated that the SPAD value and the chlorophyll content are positively correlated [38]. In this work, for ensuring the correctness of hyperspectral analysis, we use the SPAD-502 meter to directly measure the SPAD value. The change of the SPAD value can also reflect the change of the chlorophyll content accordingly [39].

In regards to measuring the SPAD value, a hand-held SPAD-502 meter, produced by KONICA MINOLTA (JAPAN), is used in the experiment. The SPAD-502 meter determines the chlorophyll concentration by measuring the leaf absorbance in red-light and near-infrared regions. Two LEDs with peak wavelengths at 650 and 940 nm emit the light. After the light passes through the sample leaf in the measuring head, the receptor will count the amount of passed light and convert them into electrical signals,

thereby calculating the SPAD value and displaying the result in the screen in real time [10]. Specifications of the SPAD-502 meter are shown in Table 4.

Tab.4 Specifications of the SPAD-502 meter	
Attribute	Value
Measurement area	2mm × 3mm
Measurement range	- 9.9 ~ 199.9 SPAD unit
Measurement duration	2 seconds
Accuracy	± 1.0 SPAD unit

The selected leaves are placed between the 2 heads of the meter for measuring the SPAD value. The duration of SPAD value measurement is the same as HSI acquisition and we measure the SPAD value for each leaf after HSIs collection. Measurement of SPAD value with SPAD-502 meter is shown in Figure 8.

Fig. 8 Measurement of SPAD using SPAD-502 meter

To ensure that the rice leaves are infected with a single disease, we inoculate leaves by artificially cutting them at their tips. In this way, the pathogen can spread along the leaf vascular tissue and infect rice leaves. The characteristics at the tip of the leaf are more obvious, so we select the ROI from the tip for our investigation. Considering the deviation caused by the difference of leaf thickness, we choose the sampling position 1 cm away from the cutoff point as the ROI, as shown in Figure 9. Wang et al [3] quantified the chlorophyll content by averaging the SPAD value of leaves in five-time sampling strategy. So averaging sampling strategy is needed for quantifying SPAD value [7]. In our experiment, because the ROI is small, we measure the SPAD value for 3 times and calculate the average value for record. Finally, we obtain 1000 pieces of SPAD value and categorize them under 6 disease levels.

Fig. 9 ROI selection diagram

Calculation of SFDI

As a matter of fact, the reflectance curve at any pixel area of a hyperspectral image can be represented by a trademark shape [40, 41]. However, this shape is considered as an irregular curve, which cannot be characterized by any numerical equation. Here, the fractal geometry is introduced. As a branch of the mathematics domain, fractal dimension is good at describing shapes, in particular, the irregular ones. The fractal dimension of any curve can be measured by its irregularity, thereby being treated as its characteristic feature [27]. It is worth mentioning that the fractal dimension can provide a comprehensive description of spatial curves. For hyperspectral data, if the spectral signals of the pixels are plotted against bands, it will generate a corresponding hyperspectral curve, which is fractal [42]. In general, the fractal dimension can be computed by the box dimension method, variance method, structure function method, covariance weighted method, divider method, power spectrum method, and wavelet transform method, and so on [28]. In this work, we compute the fractal dimension by iterating the radius. The detail of this computational method is presented in Figure 10.

Fig. 10 Flow chart of computing the spectral fractal dimension

This method follows the below 3 steps:

(1) Spectral normalization

The reflectance of calibrated hyperspectral curve ranges from 0 to 1, therefore, we adopt the maximum and minimum normalization method to eliminate the influence of wavelength magnitude. The mathematical model of wavelength normalization is formulated in Equations (2) and (3) as follows.

$$\varphi = (x_1, y_1; x_2, y_2; \dots; x_n, y_n) \quad (2)$$

$$x_i = \frac{x - x_{min}}{x_{max} - x_{min}} \quad (3)$$

Where φ denotes the pixel of the hyperspectral curve, x_n and y_n denote the n^{th} wavelength of the pre-processed spectrum, x_i denotes the wavelength after normalization, x_{min} and x_{max} are the minimum and maximum wavelength values respectively.

(2) Iteration of radius

The iteration of radius is the core of computing the fractal dimension. Because the length of hyperspectral curves can be quantified by different radius. According to the fractal theory, the minor change of hyperspectral reflectance can be detected by computing various radii. As the radius decreases, the length of the reflectance curve would become stable. The radius and the length of the curve is correlated by exponents [41]. We compute the fractal dimension of the average hyperspectral curve for each ROI by following the below sub-steps.

Sub-step 1: The reflectance curve ranging from 420 to 950 nm is read and the total number of bands is counted. The starting and end coordinates are (x_1, y_1) and (x_n, y_n) , respectively. The initial radius is defined in Equation (4).

$$r_1 = \frac{1}{2} \left(\frac{1}{n-1} \sum_{i=1}^{n-1} \sqrt{(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2} \right), (i = 1, 2, \dots, n-1) \quad (4)$$

Where r_1 denotes the initial radius.

Sub-step 2: Considering the starting coordinate (x_1, y_1) as the center of an arc, we draw an arc to intersect the hyperspectral curve. At this time, we obtain an intersection and treat it as the center of an arc for drawing the next. The drawing of arcs is repeated along the direction of the hyperspectral curve until the distance between the center of the last arc and the end coordinate (x_n, y_n) is less than the initial radius. Here, we can obtain the number of arcs, denotes as $N(r_1)$.

Sub-step 3: For further exploring the minor changes of the hyperspectral curve, we change the radius to draw the arc. The radius r_j is iterated by Equation (5), while the curve length $L(r_j)$ in each iteration

with different radii is computed by Equation (6).

$$r_{j+1} = r_j / \sqrt{2}, (j = 1, 2, \dots, M - 1) \quad (5)$$

$$L(r_j) = N(r_j) \times r_j \quad (6)$$

Where r_j denotes the radius of the j^{th} iteration, $N(r_j)$ denotes the number of arcs drew in r_j , $L(r_j)$ denotes the curve length under r_j , M is the times for termination condition of the radius iteration.

The termination condition of the radius iteration is defined that the curve length in the neighboring iteration is equal or less than r_j/M , described by Equation (7).

$$L(r_{j+1}) - L(r_j) \leq \frac{r_j}{M} \quad (7)$$

Where M denotes how many iterations are required to achieve the termination condition.

The schematic diagrams in (a), (b), and (c) of Figure 11 depict the spectral curve length measurement process for radius r_1 , r_j and r_M , respectively. It can be seen from Figure 11 that a smaller radius can more clearly reflect changes of the hyperspectral curve. The more arcs are obtained, the more accurate the curve length will be, thereby providing a more robust result [32].

Fig. 11 Measuring the curve length with radius iteration

(3) Fractal dimension logarithmic fitting

The fractal dimension of 420-950 nm bands, F_D , is fitted by the logarithmic of Hausdorff dimension [43] and the formula is defined in Equation (8).

$$F_D = -\frac{\lg(N(r_j))}{\lg(1/r_j)}, (j = 1, 2, \dots, M) \quad (8)$$

The value of the fractal dimension is the proposed index, SFDI, which lies in the interval from 1 to 2.

The SFDI is a powerful representation to reflect the complexity of hyperspectral curve [40]. The selected bands (e.g., visual and near-infrared bands) cover almost all the necessary information for estimating the SPAD value. By iterating the radius, minor changes between the reflectance of neighbor

bands can be detected and the relationship between the reflectance can be patterned. Thus, the SFDI of hyperspectral curve provides solid support for monitoring the change in the SPAD value of diseased rice leaves.

Definition and calculation of current VIs

Various researches have demonstrated the strong positive correlation between certain hyperspectral wavelengths and SPAD value [44]. In the visual region, on the one hand, the ideal wavelengths for estimating the SPAD value are near 450 and 670 nm, which correspond to the absorption peaks of SPAD value in the red and blue regions [45]. As a result, these two wavelengths are usually used as sensitive indicators to quantify the change of the SPAD value. In addition, the wavelength at 720 nm, which nears the red-edge region, is also sensitive to the absorption of SPAD value [46]. On the other hand, the wavelengths near 700 and 550 nm are often treated as anti-interference indicators because these two wavelengths correspond to the absorption valleys of SPAD value [47]. Nevertheless, the spectral region where the correlation with SPAD value under diseased leaves exceeds 0.8 is between 550-950 nm (shown in Figure 12). In the current spectral index analysis band, it is not possible to predict the SPAD value of the disease with all the relevant information. As a result, it is imperative to build an index of all spectral information. In this way, changes in photosynthetically active radiation caused by non-photosynthetic substances could be minimized or eliminated.

Fig. 12 Correlation coefficient of wavelengths and the SPAD value

Based on these sensitive bands, for quantifying and representing the SPAD value, researchers have formulated various VIs like the modified soil-adjusted vegetation index (MSAVI), normalized difference vegetation index (NDVI), photochemical reflectance index (PRI), modified chlorophyll absorption in reflective index (MCARI), etc. Some popular indices are listed in Table 5.

Tab.5 Definition of current VIs

VIs	Definition or equation	Reference
GNDVI	$(R_{800} - R_{550}) / (R_{800} + R_{550})$	[45]
MCARI	$[(R_{700} - R_{670}) - 0.2(R_{700} - R_{550})](R_{700} / R_{670})$	
VOG ₁	R_{740} / R_{720}	
VOG ₂	$(R_{737} - R_{747}) / (R_{715} + R_{726})$	[46]
VOG ₃	$(R_{737} - R_{747}) / (R_{715} + R_{720})$	
PSRI	$(R_{680} - R_{500}) / R_{750}$	
MSAVI	$\frac{1}{2} \times \left[(2R_{Nir} + 1) - \sqrt{(2R_{Nir} + 1)^2 - 8(R_{Nir} - R_{Red})} \right]$	[48]
NDVI	$(R_{800} - R_{670}) / (R_{800} + R_{670})$	[49]
PRI	$(R_{570} - R_{531}) / (R_{570} + R_{531})$	
NPCI	$(R_{680} - R_{430}) / (R_{680} + R_{430})$	
MTCI	$(R_{754} - R_{709}) / (R_{709} - R_{681})$	[51]
RVI	R_{Nir} / R_{Red}	[52]
NDI	$(R_{800} - R_{680}) / (R_{800} + R_{680})$	[53]
SAVI	$1.5 \times (R_{800} - R_{670}) / (R_{800} + R_{670} + 0.5)$	[54]
VARI _{green}	$(R_{560} - R_{670}) / (R_{560} + R_{670} - R_{450})$	[55]
VARI _{red}	$(R_{700} - 1.7R_{670} + 0.7R_{450}) / (R_{700} + 2.3R_{670} - 1.3R_{450})$	

R_{800} ——Spectral reflection intensity at 800nm, the same goes for R_{754} , R_{680} , R_{450} , and so on.

R_{Nir} —— Mean reflection intensity between 760 to 850nm.

R_{Red} ——Mean reflection intensity between 650 to 670nm.

It is acknowledged that the accuracy of SPAD prediction through current VIs would be affected by changes in spectral reflectance. Consequently, using those VIs established with few wavelengths to predict SPAD value is potentially risky. Although the combination of several wavelengths is used for index formulation, the selected wavelengths cannot fully cover the sensitive information of the SPAD value. Furthermore, the interaction of multiple wavelengths may cause saturation, thereby decreasing the accuracy of VIs. For overcoming these issues in current VIs, we propose a robust index for estimating the SPAD value of disease leaves by taking advantage of the fractal dimension of contiguous hyperspectral bands. Compared with current VIs, the newly-proposed index surely covers adequate hyperspectral information because the selected bands are wider. To further reduce the noise and improve the accuracy, bands ranging from 420 to 950 nm are selected for fractal dimension computation, because these bands show a strong positive correlation with the SPAD value and contain less noise.

Evaluation criteria

All the hyperspectral information is processed by a computer under the Windows 10 operating environment, with 16GB RAM and Inter QuadCore i7-8700 (4.2GHz). The analyzer tool is HSI Analyzer, ENVI5.3 and Matlab R2016a.

The correlation of determination (R^2), Root Mean Square Error (RMSE), and Relative Error (RE) are selected as the evaluation criteria [56]. As the R^2 value increases and the values of RMSE and RE decrease, the SPAD value prediction performed better. Formulations of evaluation criteria are defined in Equations (9-11) as follows:

$$R^2 = 1 - \frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{\sum_{i=1}^n (y_i - \bar{y})^2} \quad (9)$$

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (\hat{y}_i - y_i)^2} \quad (10)$$

$$RE = \frac{1}{n} \sum_{i=1}^n \frac{|\hat{y}_i - y_i|}{y_i} \times 100\% \quad (11)$$

Where $\hat{y}_i$ denotes the predicted value, y_i denotes the measurement, $\bar{y}$ denotes the average value of y_i , and n denotes the number of samples.

Results

Changes of spectral characteristics of disease leaves

Changes of spectral curve reflectance

Rice infected by the bacterial blight will experience a decrease in chlorophyll synthesis and photosynthesis. Furthermore, the hyperspectral reflectance characteristics of diseased rice leaves will be affected. In diseased rice leaves, the hyperspectral curve varied under different disease levels, which matched the hyperspectral characteristics of green plants. The average hyperspectral curves of the same ROI for a single leaf under 6 disease levels are shown in Figure 13.

Fig. 13 Average hyperspectral curve of ROI under different disease levels

At the early stage of bacterial blight infection, dark green water-stained streaks would appear at the

diseased region of rice leaves, while the reflectance in the visible region is increased. The reflectance from 420 to 680nm, with the disease develops further, the diseased leaves turn gray-green and becomes curled, and the reflectance further increases. Until diseased rice leaves turn yellow and white, the highest reflectance is reached. Due to the hyperspectral characteristics of the visible light region are mainly affected by the absorption of the pigment, the reflectance in the visible region is higher than that in the near-infrared region, which is supported by An et al. [57]. It is noted that the reflectance in the near-infrared region is negatively correlated with the SPAD value, while this point of view is consistent with Wang et al. [3]. It is concluded that the hyperspectral reflectance of diseased rice leaves changes with the SPAD value after bacterial blight infection, which provides a theoretical basis for the next step in analyzing the relationship between the SPAD value and the hyperspectral reflectance.

SFDI variation of spectral curve

The reflectance of the visible light region is increased with the developing disease levels, especially in the red valley region. The reflectance between 650 to 680 nm bands increases the most sharply, and shows a positive correlation with the disease stress. The increasing rate is much higher than that of between the green peak regions (525 to 575 nm). It can be concluded that SFDI is closely related to the reflectance of the hyperspectral curve. The average, maximum, minimum, and mean square error (MSE) of fractal dimension under 6 different disease levels are shown in Table 6.

Tab. 6 Statistical data of SFDI under different disease levels

Disease level	Mean	Maximum	Minimum	MSE
Level 0	1.1807	1.2042	1.1372	2.4523×10^{-4}
Level 1	1.2190	1.2408	1.1975	1.6312×10^{-4}
Level 2	1.2595	1.2895	1.2401	2.1748×10^{-4}
Level 3	1.2779	1.3036	1.2483	2.8127×10^{-4}
Level 4	1.3199	1.4126	1.2627	2.9133×10^{-4}
Level 5	1.3962	1.5040	1.3595	2.1510×10^{-4}

It can be seen from Table 4 that the fractal dimension of the average spectrum for the ROI under different disease levels has major differences. As the disease develops, the average, maximum, and maximum values of SFDI are eventually increased.

Analysis of variation of SPAD value under different disease levels

Influenced by the cell structure and leaf chlorophyll content, there are large reflective surface cavities within the spongy tissue structure of healthy rice leaf flesh, and the intracellular chlorophyll is in a hydrosol state, leading to strong infrared reflection and large SPAD value. For rice leaves under disease stress, after

the disease invaded leaves, the leaf chloroplasts and structure are damaged, which would hinder the synthesis of the leaf chlorophyll and the smooth progress of photosynthesis, and the chlorophyll content of rice leaves gradually decreases, and the SPAD value would also decrease. If rice is under serious disease stress, the SPAD value of rice leaves would drop significantly.

The SPAD value has a highly positive correlation with the chlorophyll content in plants [39]. After placing the ROI of the leaf between the 2 measuring heads, the hand-held SPAD-502 meter can show the SPAD value directly [10]. SPAD value varies with the chlorophyll content, ranging from 0 to 60, the change of SPAD value under different disease levels is shown in Figure 14.

Fig. 14 SPAD value of rice leaves under different disease levels

It can be seen from Figure 14 that the average SPAD value of diseased rice leaves show a downward trend. The average SPAD value of diseased leaf changes with the disease levels. This result is consistent with previous research that reported the chlorophyll content to decline rapidly when plants are experiencing stress and during leaf senescence [58]. In the early stage of infection, a small number of bacteria cannot cause extensive damage to leaves, and the diffusion rate is slow, so there is little change in leaf chlorophyll content in the infected area [59]. The chlorophyll content of rice leaves in level 0 is the highest, with the maximum and average SPAD value of 53.7 and 44.65, respectively. Due to the SPAD value are mainly affected by the leaf structure and the cell structure of rice leaves, both of which are damaged after bacterial blight infection. As the disease further develops, the average SPAD value gradually decreases to 11.5.

Correlation analysis between VIs and SPAD value

When using current VIs to estimate the SPAD value, it is noted that some indices will become saturated with the change of the measured parameters (e.g., the chlorophyll content). As a result, these indices would become less sensitive to changes of SPAD value under different disease levels, and eventually achieve poor accuracy. In order to determine the hyperspectral bands that are strongly correlated

with the SPAD value of rice leaves under disease stress, the correlation matrix method is used to analyze the correlation under 6 different disease levels. The correlation matrix diagram is displayed in Figure 15.

Fig.15 Correlation matrix between hyperspectral bands and SPAD value under 6 disease levels

Figure 15 shows that the SPAD value are correlated with certain hyperspectral bands under different disease levels. For instance, for the disease level 0, hyperspectral bands from 500 to 650 nm show a strong correlation with the SPAD value. For the disease level 3, a strong correlation appears at bands that range from 580 to 720 nm. Lastly, for the disease level 5, bands from 450 to 700 are positively correlated with the SPAD value. Judging from above, it can be concluded that current VIs cannot accurately estimate the SPAD value of diseased rice leaves, due to the selection of sensitive bands is usually fixed and limited. For example, NDVI only concerns the wavelength at 670 and 800 nm. Under such circumstance, NDVI may become less sensitive to the disease level 4, because the bands to be used for this disease level correspond to 480-550 nm and 620-680 nm according to our correlation analysis. The result of the statistical analysis presented in Table 7 also supports this finding. Among the 17 VIs including the SFDI, 9 VIs have a correlation coefficient with the SPAD value exceeding 0.5000, and 4 of them reach over 0.7000. The SFDI and SPAD value are correlated the strongest, the correlation coefficient reaches 0.8263.

Tab. 7 Correlations between SPAD value and VIs

VIs	correlation coefficient
SFDI	0.8263**
MSAVI	0.8024**
RVI	0.7947**

VARI _{red}	0.7235**
NPCI	0.6426*
NDVI	0.5545**
SAVI	0.4989*
GNDVI	0.4559*
VOG ₃	0.3125**
VOG ₁	-0.2692**
NDI	-0.3752**
VOG ₂	-0.4041**
PSRI	-0.4591*
PRI	-0.4779**
VARI _{green}	-0.5852**
MTCI	-0.7541**
MCARI	-0.7578**

Note: * and ** indicate that correlations are significant at confidence levels of 0.05 and 0.01, respectively.

With the further development of the disease, the rice leaves lose green and turn yellow, the chlorophyll content is decreased accordingly, and the reflectance in the visible light band is increased. The change of the chlorophyll content also leads to the change of reflectance of leaves, and at the same time change in SFDI. Therefore, the SFDI is proved being sensitive to the change of the SPAD value in disease.

Regression analysis of the SPAD value and VIs

In Table 5, VIs with a SPAD value greater than 0.7000 are selected for regression analysis. VIs under different disease levels are used as input variables, and the SPAD value to be estimated is used as an output variable. Datasets of hyperspectral images and their corresponding SPAD values are divided into training and testing sets in an 8:2 ratio. The regression models for VIs and the SPAD value are established based on 4 machine learning models, including decision tree (DT), partial least square regression (PLSR), support vector regression (SVR) and back propagation neural network (BPNN). The prediction performance of each model built with selected indices is presented in Table 8.

Tab.8 Performance of prediction models built with different VIs

VIs	Model	Training Set			Test Set		
		<i>R</i> ²	<i>RMSE</i>	<i>RE</i> /%	<i>R</i> ²	<i>RMSE</i>	<i>RE</i> /%
MSAVI	DT	0.8153	4.2358	9.3182	0.7916	4.7874	9.5617
	PLSR	0.8019	5.2545	10.2113	0.7711	5.3593	10.3156
	SVR	0.8553	4.0548	8.3935	0.8355	4.5187	9.3219
	BPNN	0.8437	3.2254	8.7417	0.8322	3.3290	10.8533
MCARI	DT	0.7215	8.3541	14.0523	0.7006	9.2147	15.3319
	PLSR	0.6853	7.4097	11.0561	0.6631	10.2416	12.1102
	SVR	0.7783	6.8345	9.7764	0.7547	10.9724	9.1542
	BPNN	0.7512	6.7714	10.2314	0.7431	9.2433	10.2011

MTCI	DT	0.5839	10.9318	20.2176	0.5581	18.5998	20.3154
	PLSR	0.6337	13.4315	17.7154	0.6255	14.3392	18.6833
	SVR	0.6239	8.3549	13.1171	0.6213	10.4582	14.6914
	BPNN	0.6617	7.9018	12.1272	0.6571	9.8851	13.2387
RVI	DT	0.5311	12.2155	19.2513	0.4924	13.5217	19.7315
	PLSR	0.5442	11.5125	18.2651	0.5351	12.3652	18.9113
	SVR	0.5537	9.2254	13.7114	0.5463	10.3290	13.8151
	BPNN	0.5329	8.7592	14.2615	0.5154	9.3651	14.3216
VARI _{red}	DT	0.7419	9.8263	10.2344	0.7224	10.2355	12.9371
	PLSR	0.7133	12.3652	14.2615	0.7062	13.6239	16.3117
	SVR	0.7939	11.9217	10.0200	0.7819	12.9759	13.8592
	BPNN	0.7785	8.8251	9.3154	0.7435	10.9472	9.8138
SFDI	DT	0.8413	4.5163	10.5127	0.8387	4.7184	10.6479
	PLSR	0.8516	3.8715	9.8435	0.8479	4.5526	9.9316
	SVR	0.8874	3.5124	7.7451	0.8752	3.7715	7.8614
	BPNN	0.8759	3.3152	8.3218	0.8679	3.6780	8.6153

From the result in Table 8, it is concluded that all regression models built with SFDI achieve better performance. For the testing set, the result of R^2 reaches over 0.8387, while the results of RMSE and RE are below 4.7500 and 10.6500% respectively. In particular, the regression model based on SVR is the optimal one, reaching R^2 , RMSE, and RE at 0.8752, 3.7715, and 7.8614% respectively.

Therefore, by using the SVR model built with SFDI, the SPAD value of diseased rice leaves can be predicted more accurately and robustly. The accuracy of the SPAD value prediction based on SFDI under 6 disease levels is shown in Figure 16.

For rice leaves under different disease levels, the SPAD value is significantly different. The higher the disease level, the lower the SPAD value. It can be seen from Figure 16 that the SVR model built with SFDI demonstrates outstanding stability and accuracy in predicting the SPAD value. The prediction performance of SPAD value under the disease level 3 is the best, the determination coefficient R^2 reaches the highest at 0.9154 and the RMSE is 3.8270. Overall, the values of R^2 and RMSE for all 6 disease levels only have a minor difference. As a result, SFDI can overcome the saturation problem of current VIs in predicting SPAD value of diseased rice leaves and provide a more robust result.

Fig. 16 Prediction results of the SVR model built with SFDI

Discussion

The influence of hyperspectral information change on VIs

In the present study, to determine the SPAD value of disease rice leaves, VIs established with hyperspectral information have been applied. As a non-destructive method for qualitative and quantitate analysis, the hyperspectral technology plays an important role. Bacterial blight would accelerate the process of chlorophyll degradation and leaf structure destruction, causing a dramatic change in the hyperspectral information, which is explained in previous study [25, 60]. In this work, SFDI has been proven to be a robust and accurate index for predicting the SPAD value of diseased rice leaves, because SFDI can better explain the complexity of hyperspectral curves. The minor changes of reflectance curves between any two adjacent hyperspectral wavelengths can be detected by SFDI through radius iteration [28]. The SFDI can comprehensively reflect the difference between the hyperspectral information of diseased rice leaves.

In the visible light region, especially green light region, the reflectance is mainly affected by leaf pigments [61]. At the early stage of disease, the chlorophyll content in the rice leaves gradually decreases, and the color of leaves change from green to yellow. The hyperspectral curve of leaves shows a green peak at around 550 nm, where leaves absorb green light at the highest rate, leading to the lowest reflectance of green light. Meanwhile, there is a red valley at around 680 nm, where the diseased leaves absorb the most red-light, resulting in the lowest red-light reflectance. As the disease progresses, chloroplasts suffered

severe damage, the SPAD value decreased, the reflectance of green and red-light regions increased [59]. The green peak and red valley disappear at this time. It is worth mentioning that the reflectance of the red-light region rises faster than that of green light region, and the original peak-valley curve gradually becomes a parabola.

It is noted that current VIs is the combination of few bands selected from hyperspectral. For instance, MSAVI is composed of near-infrared and red-light regions [48], while NDVI consists of wavelengths at 670 and 800 nm [49]. Wavelengths at 531 and 570 nm are used to establish PRI [49]. According to our analysis, the combination of different bands may have an influence on the estimation accuracy, this is consistent with the findings of Zhang [23]. On the one hand, VIs composed of more wavelengths, such as SFDI, MSAVI and RVI, would be more strongly correlated with the SPAD value under disease stress. Due to the adequate inclusion of hyperspectral information in the red-light and near-infrared regions, the correlation coefficients of these indices reach over 0.7900 with the SPAD value. On the other hand, the rest VIs that contain less hyperspectral information are all weakly correlated with the SPAD value of diseased rice leaves.

Sensitive bands under different disease levels

We also find that the SPAD value of rice leaves under different disease levels is sensitive to different bands. This is consistent with the findings in the literature [60]. According to Yu et al. [19], leaf reflectance is mainly affected by the pigment, cell structure and leaf water content in the leaf, while in the visible light waveband (350~720nm), leaf reflectance is most affected by chlorophyll. From 420 nm to 680 nm, a downward trend of the light absorption rate is shown, especially in the red valley region, the chlorophyll light absorption ability is stronger, and the reflectance shows an upward trend, same as Jin's research results [33]. A peak and a valley appear at around 550 nm and 670 nm, respectively. Meanwhile the hyperspectral reflectance between 650-700 nm raises sharply. It means that the current indices may achieve poor accuracy. For instance, NPCI [50] (with fixed wavelengths at 430 and 680 nm) may fail to estimate the SPAD value of rice leaves under the disease level 1, because the sensitive bands for this level are 550-570 nm and 710-740 nm. While the hyperspectral characteristics in the near-infrared region are mainly affected by the leaf structure and the cell structure of rice leaves, both of which are damaged after bacterial blight infection. Fortunately, the proposed index, SFDI, takes advantage of adopting a period of continuous bands ranging from 420 to 950 nm, which covers adequate hyperspectral information. The SPAD value is guaranteed to

be accurately calculated by using these information in all 6 disease levels.

Discussion about sensitive bands and various stresses

There are many causes for the change of the hyperspectral reflectance, such as insect, drought and vegetation deficiency stresses [28, 58, 61]. Plenty of studies have provided a solid foundation for the effective application of spectrum in rice disease detection and the prediction of biochemical parameters of rice leaves under various stresses [7, 23, 25], while the sensitive bands are different under various stresses. All these stresses may have potential influences on the hyperspectral characteristics, thus affecting the accuracy of the SPAD prediction. The development of new indices for SPAD prediction considering various stresses and wavelengths would be a very interesting topic to explore in future studies. Furthermore, a stable index is urgently needed to quantitatively analyze changes in rice leaf spectrum under different stresses as well as to predict biochemical parameters and detect diseases under different stresses.

Conclusion

Periodically estimating the chlorophyll content is essential to monitor the general health of rice. The ultimate objective is to maximize the crop yield and to reduce losses caused by disease spreading among crops. Current VIs (e.g., MSAVI, MCARI, MTCI, etc.) are established by considering only the sensitive (e.g., 670 and 450 nm) and anti-intervention wavelengths (e.g., 700 and 550 nm). However, the combination of selected wavelengths cannot fully reflect the minor changes of the chlorophyll content in diseased plants. Because the ignorance of certain wavelengths may lead to the loss of sensitive information and the interaction of multiple wavelengths may cause saturation, thereby decreasing the accuracy of VIs. Besides, it is found that the SPAD value of rice leaves under different disease levels is sensitive to different wavelengths. In this work, for overcoming the limitation of current VIs, a robust index is proposed by computing the fractal dimension of hyperspectral curve ranging from 420 to 950 nm. The experimental result demonstrates that the proposed index shows a stronger positive correlation with the SPAD value than current ones. We further build a SPAD prediction model by combining the proposed index with various machine learning models. The comparison result over the testing dataset shows that the prediction model based on SVR and SFDI achieves the best prediction performance, reaching R^2 , RMSE, and RE at 0.8752, 3.7715, and 7.8614%, respectively.

In conclusion, it is recommended to consider to use this newly-proposed index, SFDI, for predicting the SPAD value of diseased rice leaf with hyperspectral information ranging from 420 to 950 nm. The

prediction using SFDI is more accurate and robust. Further exploration of our study may focus on testing the SFDI over other plants to verify the applicability and usefulness of this index.

Acknowledgement

We thank the College of Plant Protection of Nanjing Agricultural University for providing the experiment materials.

Authors' contributions

CYF contributed to the conceptualization, methodology, software, data curation, writing—original draft preparation, writing—reviewing and editing. ZZY contributed to the writing-reviewing and editing, XHL and ZZY contributed to the supervision, project administration, funding acquisition. HXH contributed to the conceptualization and methodology. KTW, SJ, YY contributed to the investigation, data curation, resources. All authors read and approved the final manuscript.

Funding

The research leading to the presented results has been undertaken within the Startup Foundation of New Professor at Nanjing Agricultural University (Grant No. 106-804005), the National Natural Science Foundation of China (Grant No. 61502236), and the Fundamental Research Funds for the Central Universities (Grant No. KYLH202006 and KYZ201914).

Availability of data and materials

The remotely sensed and field sampling data used in this study is available from the corresponding author on reasonable request.

List of abbreviation

SPAD: Soil-plant analysis development
VI: Vegetation index
DT: Decision tree
PLSR: Partial least square regressions
SVR: Support vector regression
BPNN: Back propagation neural network
SFDI: Spectral fractal dimension index
HSI: Hyperspectral Image
ROI: Region of interest
SG: Savitzky-Golay
GNDVI: Green normalized difference vegetation index
MCARI: Modified chlorophyll absorption in reflective index
VOG: Vogelmann index

494 PSRI: Plant senescence reflectance index
495 MSAVI: Modified soil-adjusted vegetation index
496 NDVI: Normalized difference vegetation index
497 PRI: Photochemical reflectance index
498 NPCI: Normalized pigment chlorophyll index
499 MTCI: MERIS terrestrial chlorophyll index
500 RVI: Ratio vegetation index
501 NDI: Normalized vegetation index
502 SAVI: Soil-adjusted vegetation index
503 VARI: Visible atmospherically resistant index
504 R²: Coefficient of determination
505 RMSE: Root mean square error
506 RE: Relative error
507 MSE: Mean square error
508

509 **Declarations**

510 **Ethics approval and consent to participate**

511 All authors read and approved the manuscript.

512 **Consent for publication**

513 All authors agreed to publish this manuscript.

514 **Competing interests**

515 The authors declare that they have no known competing financial interests or personal relationships that
516 could have appeared to influence the work reported in this paper.

517 **Author details**

518 *Correspondence: zhaoyu.zhai@njau.edu.cn

519 ¹ College of Engineering, Nanjing Agricultural University, Nanjing 210032, Jiangsu, China. ² College of
520 Artificial Intelligence, Nanjing Agricultural University, Nanjing 210095, Jiangsu, China. ³ College of
521 Information Engineering, Jiangxi Vocational College of Mechanical & Electrical Technology, Nanchang
522 330013, China.

523 **References:**

- 524 1. Lu, S.; Lu, F.; You, W.; Wang, Z.; Liu, Y.; Omasa, K. A robust vegetation index for remotely assessing
525 chlorophyll content of dorsiventral leaves across several species in different seasons. *Plant Methods*. **2018**,
526 14, 1-15.
- 527 2. Yoshio, I.; Martine, G.; Frédéric, B.; Andrew, S.; Anatoly, G.; Martin, S.; Roshanak, D.; Albert, O. Simple
528 and robust methods for remote sensing of canopy chlorophyll content: a comparative analysis of
529 hyperspectral data for different types of vegetation. *Plant, Cell & Environment*. **2016**, 39, 2609-23.
- 530 3. Wang, H.; Jiang, M.; Yan, L.; Yao, Y.; Fu, Y.; Luo, S.; Lin, Y. Angular effect in proximal sensing of leaf-

level chlorophyll content using low-cost DIY visible/near-infrared camera. *Comput. Electron. Agr.* **2020**, 178, 105765-72.

4. Liang, Y.; Urano, D.; Liao, K.; Hedrick, T.L.; Gao, Y.; Jones, A.M. A nondestructive method to estimate the chlorophyll content of Arabidopsis seedlings. *Plant Methods.* **2017**, 13.
5. Berdugo, C.A.; Zito, R.; Paulus, S.; Mahlein, A.K. Fusion of sensor data for the detection and differentiation of plant diseases in cucumber. *Plant Pathol.* **2014**, 63, 1344-56.
6. Atta, B.M.; Saleem, M.; Ali, H.; Arshad, H.M.I.; Ahmed, M. Chlorophyll as a biomarker for early disease diagnosis. *Laser Phys.* **2018**, 28, 656-64.
7. Šebela, D.; Quiñones, C.; Cruz, C.V.; Ona, I.; Olejníčková, J.; Jagadish, K.S. Chlorophyll Fluorescence and Reflectance-Based Non-Invasive Quantification of Blast, Bacterial Blight and Drought Stresses in Rice. *Plant Cell Physiol.* **2018**, 59, 30-43.
8. Yang, H.; Yang, J.; Lv, Y.; He, J. SPAD Values and Nitrogen Nutrition Index for the Evaluation of Rice Nitrogen Status. *Plant Prod. Sci.* **2015**, 17, 81-92.
9. Shu, M.; Zuo, J.; Shen, M.; Yin, P.; Wang, M.; Yang, X.; Tang, J.; Li, B.; Ma, Y. Improving the estimation accuracy of SPAD values for maize leaves by removing UAV hyperspectral image backgrounds. *Int. J. Remote Sens.* **2021**, 42, 5862-81.
10. Uddling, J.; Gelang-Alfredsson, J.; Piikki, K.; Pleijel, H. Evaluating the relationship between leaf chlorophyll concentration and SPAD-502 chlorophyll meter readings. *Photosynth. Res.* **2007**, 91, 37-46.
11. Xu, X.; Gu, X.; Song, X.; Li, C.; Huang, W. Assessing Rice Chlorophyll Content with Vegetation Indices from Hyperspectral Data. Berlin, Heidelberg:Springer Berlin Heidelberg:296-303.
12. Zhao, J.; Zhang, D.; Luo, J.; Dong, Y.; Yang, H.; Huang, W. Characterization of the rice canopy infested with brown spot disease using field hyperspectral data. *Wuhan University Journal of Natural Sciences.* **2012**, 17, 86-92.
13. Yue, J.; Feng, H.; Tian, Q.; Zhou, C. A robust spectral angle index for remotely assessing soybean canopy chlorophyll content in different growing stages. *Plant Methods.* **2020**, 16.
14. Bhadra, S.; Sagan, V.; Maimaitijiang, M.; Maimaitiyiming, M.; Newcomb, M.; Shakoor, N.; Mockler, T.C. Quantifying Leaf Chlorophyll Concentration of Sorghum from Hyperspectral Data Using Derivative Calculus and Machine Learning. *Remote Sens.-Basel.* **2020**, 12, 2082-94.
15. Wang, L.; Zhou, X.; Zhu, X.; Guo, W. Estimation of leaf nitrogen concentration in wheat using the MK-SVR algorithm and satellite remote sensing data. *Comput. Electron. Agr.* **2017**, 140, 327-37.
16. Li, L.; Ren, T.; Ma, Y.; Wei, Q.; Wang, S.; Li, X.; Cong, R.; Liu, S.; Lu, J. Evaluating chlorophyll density in winter oilseed rape (*Brassica napus* L.) using canopy hyperspectral red-edge parameters. *Comput. Electron. Agr.* **2016**, 126, 21-31.
17. Bielinis, E.; Jozwiak, W.; Robakowski, P. Modelling of the relationship between the SPAD Values and Photosynthetic Pigments Content in *Quercus Petraea* and *Prunus Serotina* Leaves. *Dendrobiology.* **2015**, 73, 125-34.
18. Zhang, J.; Tian, H.; Wang, D.; Li, H.; Mouazen, A.M. A novel spectral index for estimation of relative chlorophyll content of sugar beet. *Comput. Electron. Agr.* **2021**, 184, 188-200.
19. Yu, Z.; Wang, X.; Meng, X.; Zhang, X.; Wu, D.; Liu, H.; Zhang, Z. SPAD Prediction Model of Rice Leaves Considering the Characteristics of Water Spectral Absorption. *Spectrosc. Spect. Anal.* **2019**, 39, 256-64.
20. Yang, J.; Zhang, Y.; Du, L.; Liu, X.; Shi, S.; Chen, B. Improving the Selection of Vegetation Index Characteristic Wavelengths by Using the PROSPECT Model for Leaf Water Content Estimation. *Remote Sens.-Basel.* **2021**, 13, 821-41.

21. Sun, J.; Shi, S.; Yang, J.; Gong, W.; Qiu, F.; Wang, L.; Du, L.; Chen, B. Wavelength selection of the multispectral lidar system for estimating leaf chlorophyll and water contents through the PROSPECT model. *Agr. Forest Meteorol.* **2019**, 266-267, 43-52.
22. Pane, C.; Manganiello, G.; Nicastro, N.; Cardi, T.; Carotenuto, F. Powdery Mildew Caused by *Erysiphe cruciferarum* on Wild Rocket (*Diplotaxis tenuifolia*): Hyperspectral Imaging and Machine Learning Modeling for Non-Destructive Disease Detection. *Agriculture*. **2021**, 11, 337.
23. Zhang, G.; Xu, T.; Tian, Y.; Xu, H.; Song, J.; Lan, Y. Assessment of rice leaf blast severity using hyperspectral imaging during late vegetative growth. *Australas. Plant Path.* **2020**, 49, 571-8.
24. Sun, Q.; Jiao, Q.; Qian, X.; Liu, L.; Liu, X.; Dai, H. Improving the Retrieval of Crop Canopy Chlorophyll Content Using Vegetation Index Combinations. *Remote Sens.-Basel*. **2021**, 13, 470-90.
25. Zhang, J.; Yang, Y.; Feng, X.; Xu, H.; Chen, J.; He, Y. Identification of bacterial blight resistant rice seeds using terahertz imaging and hyperspectral imaging combined with convolutional neural network. *Front. Plant Sci.* **2020**, 11, 1253-68.
26. Fuss, F.K.; Weizman, Y.; Tan, A.M. The non-linear relationship between randomness and scaling properties such as fractal dimensions and Hurst exponent in distributed signals. *Commun. Nonlinear Sci.* **2021**, 96, 879-94.
27. Ajay, K.P.; Jayanta, K.G. Quantitative Analysis of Mixed Pixels in Hyperspectral Image Using Fractal Dimension Technique. *J. Indian Soc. Remote.* **2020**, 48, 583-95.
28. Wu, L.; Liu, X.; Liu, M. Analysis of the fractal characteristic of the hyperspectral curves of the rice under plumbum pollution stress. *IEEE*, 2012, 296-300.
29. Liu, M.; Liu, X.; Ding, W.; Wu, L. Monitoring stress levels on rice with heavy metal pollution from hyperspectral reflectance data using wavelet-fractal analysis. *Int. J. Appl. Earth Obs.* **2011**, 13, 246-55.
30. Burkart, A.; Schickling, A.; Mateo, M.P.C.; Wrobel, T.J.; Rossini, M.; Cogliati, S.; Julitta, T.; Rascher, U. A Method for Uncertainty Assessment of Passive Sun-Induced Chlorophyll Fluorescence Retrieval Using an Infrared Reference Light. *IEEE Sens. J.* **2015**, 15, 4603-11.
31. Vrscay, E.R.; Otero, D.; La Torre, D. A simple class of fractal transforms for hyperspectral images. *Appl. Math. Comput.* **2014**, 231, 561-73.
32. Su, J.; Li, Y.; Hu, Q. A New Spectral – Spatial Jointed Hyperspectral Image Classification Approach Based on Fractal Dimension Analysis. *Fractals*. **2019**, 27, 1524-36.
33. Jin, X.; Lu, J.; Wang, S.; Qi, H.; Li, S. Classifying wheat hyperspectral pixels of healthy heads and fusarium head blight disease using a deep neural network in the wild field. *Remote Sens.-Basel*. **2018**, 10, 395-410.
34. Ruffin, C.; King, R.L.; Younani, N.H. A combined derivative spectroscopy and Savitzky-Golay filtering method for the analysis of hyperspectral data. *Gisci. Remote Sens.* **2008**, 45, 1-15.
35. Zhou, Q.; Huang, W.; Fan, S.; Zhao, F.; Liang, D.; Tian, X. Non-destructive discrimination of the variety of sweet maize seeds based on hyperspectral image coupled with wavelength selection algorithm. *Infrared Phys. Techn.* **2020**, 109, 103418.
36. Huang, Y.; Li, J.; Yang, R.; Wang, F.; Li, Y.; Zhang, S.; Wan, F.; Qiao, X.; Qian, W. Hyperspectral Imaging for Identification of an Invasive Plant *Mikania micrantha* Kunth. *Front. Plant Sci.* **2021**, 12.
37. Pi, W.; Du, J.; Bi, Y.; Gao, X.; Zhu, X. 3D-CNN based UAV hyperspectral imagery for grassland degradation indicator ground object classification research. *Ecol. Inform.* **2021**, 62, 101278.
38. Ling, Q.; Huang, W.; Jarvis, P. Use of a SPAD-502 meter to measure leaf chlorophyll concentration in *Arabidopsis thaliana*. *Photosynth. Res.* **2011**, 107, 209-14.
39. Yuan, Z.; Ata-Ul-Karim, S.T.; Cao, Q.; Lu, Z.; Cao, W.; Zhu, Y.; Liu, X. Indicators for diagnosing nitrogen status of rice based on chlorophyll meter readings. *Field Crop. Res.* **2016**, 185, 12-20.

40. Kriti, M.; Jayanta, K.G.; Ramesh, C.M. Dimensionality reduction of hyperspectral data using spectral fractal feature. *Geocarto Int.* **2012**, *27*, 108-16.
41. Hosseini, S.A.; Ghassemian, H. A new hyperspectral image classification approach using fractal dimension of spectral response curve. *Electrical Engineering (ICEE), 2013 21st Iranian Conference on:IEEE*, 2013, 1-6.
42. Sahoo, B.; Routray, K.L.; Asokan, K.; Behera, D. Study of fractal dimension and power spectral density analysis of superconductor/ferromagnetic bilayer. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms.* **2018**, *433*, 51-9.
43. Husain, A.; Reddy, J.; Bisht, D.; Sajid, M. Fractal dimension of coastline of Australia. *Sci. Rep.-UK.* **2021**, *11*, 537-47.
44. Dong, T.; Shang, J.; Chen, J.M.; Liu, J.; Qian, B.; Ma, B.; Morrison, M.J.; Zhang, C.; Liu, Y.; Shi, Y.; *et al.*. Assessment of Portable Chlorophyll Meters for Measuring Crop Leaf Chlorophyll Concentration. *Remote Sens.-Basel.* **2019**, *11*, 2706-26.
45. Daughtry, C.; Walthall, C.L.; Kim, M.S.; de Colstoun, E.B.; McMurtrey, J.E. Estimating Corn Leaf Chlorophyll Concentration From Leaf and Canopy Reflectance. *Remote Sens. Environ.* **2000**, *74*, 229-39.
46. Vogelmann, J.E.; Rock, B.N.; Moss, D.M. Red edge spectral measurements from sugar maple leaves. *Int. J. Remote Sens.* **2007**, *14*, 1563-75.
47. Merzlyak, M.N.; Gitelson, A.A.; Chivkunova, O.B.; Rakitin, V.Y. Non-Destructive Optical Detection of Pigment Changes During Leaf Senescence and Fruit Ripening. *Physiol. Plantarum.* **1999**, *106*, 135-41.
48. Guerra, F.; Puig, H.; Chaume, R. The forest-savanna dynamics from multi-date Landsat-TM data in Sierra Parima, Venezuela. *Int. J. Remote Sens.* **1998**, *19*, 2061-75.
49. Gamon, J.A.; Penuelas, J.; Field, C.B. A Narrow-Waveband Spectral Index That Tracks Diurnal Changes in Photosynthetic Efficiency. *Remote Sens. Environ.* **1992**, *41*, 35-44.
50. Penuelas, J.; Gamon, J.A.; Griffin, K.L.; Field, C.B. Assessing Community Type, Plant Biomass, Pigment Composition, and Photosynthetic Efficiency of Aquatic Vegetation from Spectral Reflectance. *Remote Sens. Environ.* **1993**, *46*, 110-8.
51. Dash, J.; Curran, P.J. Evaluation of the MERIS terrestrial chlorophyll index (MTCI). *Adv. Space Res.* **2007**, *39*, 100-4.
52. Pinter, P.J.; Zipoli, G.; Maracchi, G.; Reginato, R.J. Influence of Topography and Sensor View Angles on Nir Red Ratio and Greenness Vegetation Indexes of Wheat. *Int. J. Remote Sens.* **1987**, *8*, 953-7.
53. Sims, D.A.; Gamon, J.A. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sens. Environ.* **2002**, *81*, 337-54.
54. Rondeaux, G.; Steven, M.; Baret, F. Optimization of soil-adjusted vegetation indices. *Remote Sens. Environ.* **1996**, *55*, 95-107.
55. Gitelson, A.A.; Stark, R.; Grits, U.; Rundquist, D.; Kaufman, Y.; Derry, D. Vegetation and soil lines in visible spectral space: A concept and technique for remote estimation of vegetation fraction. *Int. J. Remote Sens.* **2010**, *23*, 2537-62.
56. Wang, S.; Cheng, X.; Zheng, D.; Song, H.; Han, P.; Yuen, P. Prediction of the Soil Organic Matter (SOM) Content from Moist Soil Using Synchronous Two-Dimensional Correlation Spectroscopy (2D-COS) Analysis. *Sensors-Basel.* **2020**, *20*, 2351-65.
57. An, G.; Xing, M.; He, B.; Liao, C.; Huang, X.; Shang, J.; Kang, H. Using Machine Learning for Estimating Rice Chlorophyll Content from In Situ Hyperspectral Data. *Remote Sens.-Basel.* **2020**, *12*, 3104.
58. Salehi, A.; Tasdighi, H.; Gholamhoseini, M. Evaluation of proline, chlorophyll, soluble sugar content and uptake of nutrients in the German chamomile (*Matricaria chamomilla* L.) under drought stress and organic

fertilizer treatments. *Asian Pacific Journal of Tropical Biomedicine*. **2016**, 6, 886-91.

59. Zhu, M.; Yang, H.; Li, Z. Early Detection and Identification of Rice Sheath Blight Disease Based on Hyperspectral Image and Chlorophyll Content. *Spectrosc. Spect. Anal.* **2019**, 39, 1898-904.

60. Das, p.k.; Laxman, B.; Rao, S.V.C.K.; Seshasai, M.V.R.; Dadhwal, V.K. Monitoring of bacterial leaf blight in rice using ground-based hyperspectral and LISS IV satellite data in Kurnool, Andhra Pradesh, India. *Int. J. Pest Manage.* **2015**, 61, 359-68.

61. Zhang, H.; Duan, Z.; Li, Y.; Zhao, G.; Zhu, S.; Fu, W.; Peng, T.; Zhao, Q.; Svanberg, S.; Hu, J. Vis/NIR reflectance spectroscopy for hybrid rice variety identification and chlorophyll content evaluation for different nitrogen fertilizer levels. *Royal Society open science*. **2019**, 6, 2102-15.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Responsereportfor2reviewers.docx](#)