Postprint: Early Detection of Rice Leaf Blast Disease Based on Solar-Induced Chlorophyll Fluorescence Information from Different Leaf Positions

Authors: Cheng Yuxin, Xue Bowen, Kong Yuanyuan, Yao Dongliang, Tian Long, Wang Xue, Yao Xia, Zhu Yan, Cao Weixing, Cheng Tao

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Abstract

[Purpose/Significance] Non-destructive early monitoring of Rice Leaf Blast (RLB) based on remote sensing plays an important role in resistance breeding and plant protection. Current research on rice blast mostly uses reflectance spectroscopy for monitoring at its symptomatic stage, and studies on Solar-Induced Chlorophyll Fluorescence (SIF) spectral monitoring for the early infection stage of rice leaf blast have not been reported. The objective of this study is to achieve accurate identification of infected leaves at the early stage of rice leaf blast based on SIF information from different leaf positions. [Methods] Based on one-year greenhouse inoculation experiments and field sampling experiments, and using an active light source, ASD (Analytical Spectral Devices) field spectrometer, and FluoWat leaf clip in combination, leaf SIF spectra from top 1st to top 4th leaf positions of rice plants at jointing and heading stages were obtained, and disease severity levels of the tested samples were manually labeled. The study extracted wavelet features sensitive to rice leaf blast based on Continuous Wavelet Analysis (CWA), compared the sensitive features and identification accuracy of infected leaves among different leaf positions, and finally constructed a rice leaf blast identification model based on the Linear Discriminant Analysis (LDA) algorithm. [Results and Discussion] The upwelling and downwelling SIF in the far-red region of infected leaves at all leaf positions were significantly higher than those of healthy leaves; the identification accuracy of infected leaves based on SIF wavelet features was significantly higher than that of original SIF bands, and the identification accuracy of rice blast for the top 1st leaf was significantly higher than that of the other three leaf positions, with the highest accuracy reaching 70%; the extracted common sensitive wavelet features applicable to multiple leaf positions, ↑WF832,3 and ↓WF809,3, achieved accuracies of 69.45%, 62.19%, 60.35%, 63.00% and 69.98%, 62.78%, 60.51%, 61.30% for top 1st to top 4th leaves, respectively. [Conclusion] This study revealed the response patterns of rice leaf SIF spectra under rice blast stress, extracted SIF wavelet features sensitive to rice leaf blast, and the results demonstrated the potential of continuous wavelet analysis and SIF technology for diagnosing rice leaf blast, providing important reference and technical support for achieving early, rapid, and in-situ diagnosis of rice blast in the field.

Full Text

Spectroscopic Detection of Rice Leaf Blast Infection at Different Leaf Positions at The Early Stages With Solar-Induced Chlorophyll Fluorescence

CHENG Yuxin, XUE Bowen, KONG Yuanyuan, YAO Dongliang, TIAN Long, WANG Xue, YAO Xia, ZHU Yan, CAO Weixing, CHENG Tao*

(National Engineering and Technology Center for Information Agriculture (NETCIA) / Jiangsu Key Laboratory for Information Agriculture / MARA Key Laboratory of Crop System Analysis and Decision Making / MOE Engineering Research Center of Smart Agriculture, Nanjing Agricultural University, Nanjing 210095, China)

Abstract

[**Objective**] Non-destructive early monitoring of rice leaf blast (RLB) using remote sensing plays a crucial role in resistance breeding and plant protection. Current studies on rice blast primarily utilize reflectance spectroscopy during symptomatic stages, while research on solar-induced chlorophyll fluorescence (SIF) spectroscopy for monitoring early-stage RLB infection remains unreported. This study aims to accurately identify infected leaves at early stages of rice leaf blast based on SIF information from different leaf positions.

[**Methods**] Based on one-year greenhouse inoculation experiments and field sampling trials, SIF spectra of leaves at the top 1st to 4th positions of rice plants at jointing and heading stages were acquired using an active light source, an Analytical Spectral Devices (ASD) spectrometer, and a FluoWat leaf clip. Disease severity levels of measured samples were manually annotated. The study extracted wavelet features sensitive to RLB based on continuous wavelet analysis (CWA), compared the sensitive features and their identification accuracy for infected leaves across different leaf positions, and finally constructed RLB identification models using linear discriminant analysis (LDA) algorithms.

[**Results and Discussion**] The upward and downward SIF in the far-red region of infected leaves at each leaf position were significantly higher than those of healthy leaves. The identification accuracy of infected leaves based on wavelet features was significantly higher than that based on original SIF bands. The identification accuracy for the top 1st leaf was significantly higher than for the other three leaf positions, reaching up to 69.45%. The common sensitive wavelet features extracted for multiple leaf positions achieved accuracies of 62.19%, 63.00%, 60.35%, and 69.98% for the top 1st to 4th leaves, respectively.

[**Conclusion**] This study revealed the SIF spectral response patterns of rice leaves under rice blast stress, extracted wavelet features sensitive to rice leaf blast, and demonstrated the potential of continuous wavelet analysis and SIF technology for diagnosing rice leaf blast, providing important reference and technical support for early, rapid, and in-situ diagnosis of rice blast in the field.

Keywords: rice leaf blast (RLB); solar-induced chlorophyll fluorescence (SIF); continuous wavelet analysis (CWA); leaf position; early stage disease detection

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Rice blast, caused by the fungal pathogen *Magnaporthe oryzae*, is the most destructive disease affecting rice production globally. In China, the average annual occurrence area of rice blast exceeded 3 million hectares between 2010 and 2020, causing annual yield losses of hundreds of thousands of tons [1]. Due to its high infectivity, numerous conidia spread from infected leaf tissues through wind and water under conditions of insufficient light and high humidity [2, 3]. Among all types of rice blast, panicle blast has the most severe impact on yield, and studies have demonstrated that most spores causing panicle and neck blast originate from infected leaf tissues [4]. Therefore, accurate monitoring and control of rice leaf blast during its early infection stage can effectively prevent disease spread and reduce the risk of epidemics and yield loss.

Traditional rice blast surveys rely on visual inspection of symptoms by plant protection specialists to determine disease severity. However, visual assessment is subjective, time-consuming, labor-intensive, and cannot achieve presymptomatic early detection. In recent years, remote sensing technology has been applied for disease monitoring at various spatial scales, enabling detection of rice blast and identification of infection severity. However, these studies have primarily focused on symptomatic stages, lacking spectral monitoring during early infection phases that are critical for resistance breeding and plant protection [5, 6].

Since rice blast typically initiates from the middle and lower canopy through wind and water transmission [7], leaf position analysis is important for improving early monitoring accuracy. Previous research indicates that leaves at different positions exhibit different physiological states due to varying environmental conditions and developmental processes [8, 9]. Most existing studies use reflectance spectroscopy for crop disease monitoring [10, 11], but reflectance primarily reveals biochemical composition information and cannot directly reflect photosynthetic physiological status. Compared with reflectance spectroscopy, chlorophyll fluorescence is directly related to photosynthesis and can detect physiological changes caused by disease earlier and more sensitively [12].

Chlorophyll fluorescence detection is categorized into active and passive methods. Passive fluorescence detection measures solar-induced chlorophyll fluorescence (SIF) emitted by vegetation under natural light conditions. Unlike active fluorescence techniques [13], SIF directly reflects dynamic changes in actual photosynthesis and is considered a direct probe for monitoring vegetation physiological status, providing earlier and more sensitive methods for accurate diagnosis of vegetation disease stress. SIF is suitable for large-area, non-destructive monitoring of vegetation in natural environments [14]. Zhao et al. [15] compared the sensitivity of SIF, vegetation indices, and derivative spectral indices for monitoring wheat stripe rust severity at different infection stages and constructed monitoring models. Yan et al. [16] used normalized difference vegetation index (NDVI) and re-normalized vegetation index (RDVI) to process SIF and photochemical reflectance index (PRI), constructing a synergistic spectral index of SIF and PRI (SISP) that improved remote sensing monitoring accuracy of wheat stripe rust at the canopy level. However, previous studies have primarily used 1-2 bands of fluorescence information retrieved at the canopy scale, and no research has been reported on using SIF spectroscopy for rice blast diagnosis.

In terms of spectral analysis methods, most studies have used SIF yield indices [17, 18], which cannot fully utilize detailed information in leaf SIF spectra. As an effective signal processing tool, continuous wavelet analysis can capture weak spectral signals and has been gradually applied in extracting sensitive bands for crop growth parameters and diseases [19, 20]. Using continuous wavelet methods to extract RLB-sensitive SIF features can fully exploit absorption characteristics of certain crop physiological and biochemical parameters, which is important for improving early RLB monitoring accuracy and mechanistic understanding. This study aims to extract RLB-sensitive SIF features using continuous wavelet analysis based on SIF spectra from different leaf positions to achieve early diagnosis of rice blast in the field. The main research contents include: (1) clarifying the response patterns of rice SIF spectra under rice blast stress; (2) extracting RLB-sensitive SIF features and constructing identification models; and (3) comparing the accuracy differences of RLB identification models across different leaf positions to clarify the variation pattern of identification accuracy with leaf position.

2.1 Experimental Design

2.1.1 Experimental Settings Greenhouse and field experiments were conducted during the rice growing season from July to August 2021. Greenhouse experiments offer advantages of controllable environmental conditions, effective artificial inoculation, and continuous monitoring of infected plants, while field experiments provide growth conditions closer to actual production and access to leaves at all disease severity levels. Previous studies have shown that rice is most susceptible to blast during the jointing and early heading stages [21]. However, due to limitations in greenhouse and field conditions, data for both growth stages could not be obtained under both conditions. The specific experimental settings were as follows:

Greenhouse pot experiments were conducted in an intelligent greenhouse at Nanjing Agricultural University in Nanjing, Jiangsu Province (118°50'E, 32°2'N). Three japonica rice varieties common in Jiangsu Province were used: Yangnongdao 1 (highly susceptible to blast), and Nangeng 5055 and Nangeng 9108 (conventional varieties). After seedling cultivation, the three rice varieties were transplanted into plastic pots (35 cm diameter, 32 cm height, approximately 28 L volume) with 3-4 seedlings per pot. Each variety had 12 pots (6 for inoculation group and 6 for control group), totaling 72 pots. The nutrient management in the greenhouse experiment was consistent with field cultivation: urea (300 kg/ha) and compound fertilizer (150 kg/ha) were applied as basal fertilizer three days after transplanting, and a thin water layer was maintained to ensure soil moisture. Two healthy tillers were selected per pot, and the top 1st to 4th leaves on each tiller were tagged for continuous spectral measurements. A total of 77 effective samples were obtained at the jointing stage in the greenhouse, as shown in Table 1 .

Field experiments were conducted at a family farm in Duntang Village, Tongzhou District, Nantong City, Jiangsu Province (121°5'E, 32°7'N), where the rice variety Yangnongdao 1 was planted. At the heading stage, healthy and infected rice plants were selected from the diseased paddy field, and spectral measurements were performed on the top 1st to 4th leaves. A total of 66 effective samples were obtained, as shown in Table 1.

Table 1 Sets of greenhouse and field experiments and samples

Note: L1-L4 represent the top 1st to 4th leaves, respectively.

2.1.2 Artificial Inoculation and Disease Severity Definition At the tillering stage, the inoculation group in the greenhouse was treated with rice blast inoculation. The spore solution used for inoculation was provided by the State Key Laboratory of Crop Genetics and Germplasm Enhancement at Nanjing Agricultural University, including four different races of *Magnaporthe oryzae*. The inoculation group was uniformly sprayed with a spore solution at a concentration of 0.09 mg/mL, while the control group was sprayed with an equal amount of clean water. After inoculation, the rice plants were immediately covered with black opaque plastic pads. The intelligent greenhouse consisted of two layers of transparent material and was equipped with air conditioning and shading facilities, providing suitable environmental conditions for the pathogen. After 48 hours of treatment at 26-32°C, relative humidity above 90%, and no light, the plastic pads were removed.

To standardize the description of different infection stages, reference was made to the Rice Blast Survey Standard (GB/T 15790-2009). Based on lesion size and condition, infected leaves were classified into six grades, as shown in Table

2 . Grade 1 and Grade 2 represent early stages of rice leaf blast infection. The disease severity level of each leaf sample was confirmed through visual inspection of lesion symptoms. RGB images of rice leaf samples under different health conditions are shown in Figure 1 [Figure 1: see original paper].

Table 2 Criteria for classification of rice leaf blast

Grade	Description
0	No lesions or only a few pinhole-like lesions
1	Few small lesions (lesion number <5, length less than 1/2 leaf width)
2	Many small lesions or a few large lesions
3	Many large lesions
4	Lesions causing functional leaf death
5	Complete leaf death

2.2 Instruments and Data Collection

2.2.1 Experimental Instruments This study used a Spectralon reflectance standard (ODM-98, Gigahertz-Optik GmbH, Türkenfeld, Germany) to measure incident solar radiation. The FluoWat leaf clip (Producción Por Mecanizados Villanueva S.L.U, Spain) had optical fibers that could be vertically positioned on the upper and lower surfaces of leaves. When a leaf was clamped in the leaf clip, the operator could manually position the incident light at a 45° angle relative to the leaf surface through an opening in the leaf clip to measure SIF spectra. Incident light with wavelengths greater than 650 nm was cut off by a high-performance low-pass filter set at the opening of the leaf clip, allowing upward and downward SIF emitted from the leaf to be measured separately at the same position on the upper and lower surfaces of rice leaves. The measurement principle of the FluoWat leaf clip is shown in Figure 2(a) [Figure 2: see original paper].

Spectral measurements were conducted using a FieldSpec4 hyperspectral spectrometer (Analytical Spectral Devices Inc., USA), a FluoWat leaf clip, and an HLG-150 halogen lamp (Qingxuan Technology Co., Ltd., China) to measure leaf chlorophyll fluorescence spectra (Figure 2(b) [Figure 2: see original paper]). The ASD spectrometer had a wavelength range of 350-2500 nm, with a spectral sampling interval of 1.4 nm and spectral resolution of 3 nm for 350-1000 nm, and a sampling interval of 2 nm and resolution of 10 nm for 1000-2500 nm. A fiber optic cable (1.5 m) connected the FieldSpec4 Pro spectrometer (FOV = 25°) to the FluoWat leaf clip to capture light reflected from the target.

2.2.2 SIF Spectral Acquisition In both greenhouse and field experiments, white reference calibration and ASD optimization were performed after every 10 plant measurements. For each rice plant, the top 1st to 4th leaves were selected for spectral collection. For each leaf, upward and downward fluorescence were measured at three positions (1/3, 1/2, and 2/3 of the distance from the leaf base), avoiding the main leaf vein. Three replicate spectra were acquired at each measurement point. The nine upward and nine downward SIF spectra collected from the same leaf were averaged to represent the upward and downward SIF values for that leaf, effectively reducing random errors during measurement.

2.3 Analysis Methods

2.3.1 Continuous Wavelet Spectral Analysis Vegetation SIF spectral analysis employed continuous wavelet transform (CWT) as the core method. By convolving the SIF spectrum with translated and scaled mother wavelet functions, wavelet coefficients at various scales were obtained [19], as shown in Equations (1) and (2):

$$\psi_{a,b}(\lambda) = \frac{1}{\sqrt{a}}\psi\left(\frac{\lambda - b}{a}\right)$$

$$WF_{a,b} = \langle f, \psi_{a,b} \rangle = \int_{l_1}^{l_2} f(\lambda)\psi_{a,b}(\lambda)d\lambda$$

where $\psi(\lambda)$ is the mother wavelet function; λ is the spectral wavelength; a is the scaling factor (scale); b is the translation factor; $WF_{a,b}$ is the wavelet coefficient (also called wavelet feature, representing the similarity between the mother wavelet function and the original SIF value at scale a and translation factor b); $\psi_{a,b}(\lambda)$ is the translated and scaled mother wavelet function; and l_1 and l_2 are the starting and ending wavelengths of the input SIF spectrum.

The second derivative of the Gaussian function, also known as the Mexican Hat (Mexh) wavelet, was used as the mother function for continuous wavelet transform due to its shape similarity to absorption valleys and peaks in crop SIF spectra. Using the second derivative of the Gaussian function for spectral transformation is equivalent to smoothing the SIF spectrum with a Gaussian function before calculating the second derivative, which removes noise and smooths the data [22-24]. Therefore, this study adopted the second derivative of the Gaussian function as the mother wavelet for analyzing leaf SIF spectra. Figure 3 [Figure 3: see original paper] shows the upward SIF spectrum of a healthy top 4th leaf sample (Figure 3(a)), wavelet coefficients at different scales (Figure 3(b)), and the classification scalogram for RLB-sensitive features (Figure 3(c)), where the red area represents the top 1% most sensitive wavelet features. Scales 3, 4, 5, and 6 (Scale3, Scale4, Scale5, Scale6) used in this study are abbreviations for scaling factors 2^3, 2^4, 2^5, and 2^6, respectively.

2.3.2 Model Development and Validation The ability of spectral features to distinguish between infected and healthy leaf samples based on specific thresholds is called separability [20]. This study employed linear discriminant analysis (LDA) to evaluate the separability of SIF features and construct RLB identification models [25, 26]. Since this study aimed to achieve early monitoring of rice blast, Grade 0 samples were used as the healthy dataset, and Grade 1 and Grade 2 samples were used as the infected dataset for subsequent analysis. Sensitive features were selected from SIF spectra and multi-scale continuous wavelet-transformed SIF wavelet coefficients. The identification accuracy of infected leaves was compared across optimal features for each leaf position, common features across multiple leaf positions, and commonly used canopy SIF monitoring features (F760) [18]. Classification models were developed using 70% of samples for training and 30% for validation. Model accuracy was evaluated using 4-fold cross-validation repeated 100 times, with the average overall accuracy (OA) serving as the final evaluation metric. The technical route of this study is shown in Figure 4 [Figure 4: see original paper]. All operations were implemented in Python 3.9.

3 Results and Analysis

3.1 SIF Spectral Comparison

Figure 5 [Figure 5: see original paper] shows the upward and downward SIF spectra of healthy and infected leaf samples from the top 1st to 4th leaves. Upward SIF from leaves at all positions exhibited a bimodal characteristic, with peaks in the red region near 690 nm and in the far-red region near 740 nm. The red region peak was lower than the far-red region peak. Downward SIF from all leaf positions showed a unimodal characteristic, with the peak in the far-red region near 740 nm. For any given leaf position, the downward SIF signal was weaker than the upward SIF.

As shown in Figure 5, the upward and downward SIF in the far-red region of infected leaves at each leaf position were significantly higher than those of healthy leaves. However, in the red region, infected samples from the top 2nd and 4th leaves showed higher SIF than healthy samples, while the SIF values of infected and healthy samples from the top 1st and 3rd leaves were essentially the same. Additionally, both upward and downward SIF in the red and far-red regions increased with decreasing leaf position.

3.2 Selection of RLB-Sensitive Features Based on Upward and Downward SIF Separability

Figure 6 [Figure 6: see original paper] presents the classification scalograms for RLB-sensitive features obtained through separability analysis of upward and downward SIF from healthy and infected samples at different leaf positions. Sensitive features were generally dispersed. Sensitive features extracted from upward SIF were mainly distributed in the 665-680 nm and 815-830 nm regions (Figures 6(a)-6(d)), while those from downward SIF were distributed in the 665-680 nm and 755-790 nm regions (Figures 6(e)-6(h)). Thus, 665-680 nm, 755-790 nm, and 815-830 nm were identified as three major RLB-sensitive regions, with 665-680 nm being a common sensitive region for both upward and downward

SIF.

As shown in Figure 6, sensitive wavelet features also varied among leaf positions. For the top 1st leaf, sensitive features were concentrated in the 665-680 nm, 755-790 nm, and 815-830 nm regions (Figures 6(a) and 6(e)). For the top 2nd leaf, sensitive features were distributed in the 665-680 nm and 815-830 nm regions (Figures 6(b) and 6(f)). For the top 3rd leaf, sensitive features appeared at 690 nm, 755-790 nm, and 815-830 nm (Figures 6(c) and 6(g)), with sensitive bands around 690 nm observed only for this leaf position. For the top 4th leaf, sensitive features were primarily located at 665-680 nm, 725 nm, and 815-830 nm (Figures 6(d) and 6(h)), with sensitive bands around 725 nm observed only for this leaf position. Consequently, most sensitive features at each leaf position were located on the steep slopes of the SIF spectrum and mainly distributed at wavelet scales 3, 4, and 5.

Figure 7 [Figure 7: see original paper] shows the distribution of common sensitive features across the four leaf positions. The distribution pattern of common sensitive features was generally consistent with that of individual leaf positions. Common sensitive features from upward SIF were distributed in the 665-680 nm, 725 nm, and 815-830 nm regions (Figure 7(a)), while those from downward SIF were concentrated in the 665-680 nm, 690 nm, and 755-790 nm regions (Figure 7(b)). As shown in Figure 7, sensitive features remained concentrated on the steep slopes of the SIF spectrum, with better classification performance observed near 675 nm and 815 nm.

3.3 RLB Identification Based on Sensitive SIF Wavelet Features

Table 3 presents the optimal features and corresponding overall classification accuracies for each leaf position based on upward and downward SIF. The classification accuracy of sensitive features extracted from upward SIF was generally higher than that from downward SIF, most notably for the top 3rd leaf samples (Figure 8 [Figure 8: see original paper]). The optimal features and model classification accuracies varied among leaf positions. Based on upward SIF, the optimal feature for the top 1st leaf was WF815,3, achieving an overall classification accuracy above 70%, significantly higher than the other three leaf positions. The validation accuracies for the top 2nd, 3rd, and 4th leaves were between 63%-65%, with optimal features of WF672,5, WF819,3, and WF676,6, respectively. The optimal features based on downward SIF for the four leaf positions were WF759,4, WF678,4, WF758,3, and WF678,4, respectively, with the top 1st leaf model accuracy again significantly higher than other leaf positions.

Table 3 Overall classification accuracy of healthy and infected samples for different leaf positions based on upward and downward SIF and different SIF features

Leaf Position	Upward SIF Optimal Feature	Upward SIF Accuracy (%)	Downward SIF Optimal Feature	Downward SIF Accuracy (%)	Common Feature WF832,3	Common Feature WF809,3
L1	WF815,3	70.13	WF759,4	70.90	69.45	69.98
L2	WF672,5	63.70	WF678,4	63.12	62.19	62.78
L3	WF819,3	64.63	WF758,3	62.00	60.35	60.51
L4	WF676,6	64.53	WF678,4	64.02	63.00	61.30

Note: L1-L4 represent the top 1st to 4th leaves; WFa,b represents the wavelet feature at wavelength a and scale b.

Figure 9 [Figure 9: see original paper] compares the monitoring model accuracies for different leaf positions based on optimal features, common features, and the commonly used canopy monitoring feature (F760). Overall, the classification accuracy ranked as: optimal features > common features > F760. However, for the top 1st leaf dataset, the overall classification accuracies of the three feature types were all around 70%, showing essentially equivalent performance. For the other three leaf positions, the validation accuracy of common features was slightly lower than that of optimal features, while the monitoring accuracy of F760 was significantly lower than both optimal and common features.

4 Discussion

4.1 Advantages of Continuous Wavelet Spectral Analysis

Compared with original SIF features, sensitive wavelet features can effectively improve the identification accuracy of infected leaves. When applied to vegetation reflectance spectral analysis, continuous wavelet analysis can decompose individual spectral signals into wavelet coefficients at different scales. Compared with traditional analysis methods, this approach more effectively utilizes spectral interval information and overall structural characteristics of spectra [27, 28]. Through continuous wavelet analysis, this study found that wavelet features in the common sensitive region of 665-680 nm for both upward and downward SIF not only have physiological significance but also coincide with chlorophyll absorption peaks, allowing for reasonable spectral interpretation. Additionally, low-scale SIF wavelet features showed higher identification accuracy for rice leaf blast. Previous research indicates that low-scale wavelet coefficients primarily contain high-frequency information from the original spectrum, while high-scale coefficients mainly contain low-frequency information. Therefore, low-scale coefficients better reflect spectral noise and minor absorption features, whereas high-scale coefficients better reflect obvious absorption features and leaf structural changes [29, 30]. The common features WF832,3 and WF809,3 extracted in this study were both distributed at scale 3, indicating that infected leaves at all leaf positions may have similar SIF waveform characteristics in the 800-840 nm region, possibly related to cell structure destruction by *Magnaporthe oryzae*.

4.2 Mechanism of SIF Spectral Response

The results showed that upward and downward SIF in the far-red region of infected leaves at each leaf position were significantly higher than those of healthy leaves. Previous studies indicate that factors affecting plant SIF spectra mainly include environmental factors and plant biochemical parameters [31]. Since experiments in this study were conducted under the same environmental conditions, the main factors causing spectral differences between infected and healthy leaves were plant physiological and biochemical parameters such as leaf structure and photosynthetic pigment content. The *Magnaporthe oryzae* pathogen invades leaf mesophyll tissue by destroying epidermal cells and gradually penetrates cell walls to facilitate hyphal spread between cells [20, 32]. This infection process destroys chloroplast structure, leading to deterioration of plasma membrane and mitochondrial ultrastructure, damage to photosystems, and inhibition of photosystem photochemical activity. This affects electron transfer from Photosystem II (PSII) reaction centers to plastoquinone (PQ), inhibiting the primary reaction of photosynthesis, which may be one of the main reasons for SIF spectral changes. Additionally, studies have shown that *Magnaporthe oryzae* infection also leads to decreased chlorophyll a content, reducing light absorption and transfer efficiency [33, 34], which may be another main reason for enhanced SIF signals in infected leaves.

This study also found that the disease monitoring capability of the top 1st leaf was significantly higher than that of the other three leaf positions, and the optimal features extracted from the four leaf positions were all different. This may be due to differences in the physiological states of the four leaf positions at the time of monitoring. Previous studies have proven that rice leaves at different positions have different physiological performance [9], and this vertical heterogeneity within the canopy can be viewed as an adaptive strategy for continuously adjusting resource allocation within and between leaves to maximize canopy photosynthetic rates in response to limited nutritional resources and fluctuating environmental conditions [35, 36]. Although some top 3rd and 4th leaves had gradually entered the senescence stage, leaf senescence causes chlorophyll content reduction and photosynthetic capacity decline [37]. Therefore, both rice blast stress and senescence may cause SIF spectral changes in middle and lower leaves, affecting the identification accuracy of SIF features for infected leaves at these positions. The top 1st leaf is the newest unfolding leaf at the uppermost part of the rice plant, basically in the leaf expansion stage. According to plant nutrition theory, nutrients are preferentially supplied to growth points, fruits, and new leaves. Zhu et al. [37] also demonstrated that the correlation between chlorophyll fluorescence parameters and reflectance spectral characteristics was highest in wheat top leaves. Therefore, the top 1st leaf SIF dataset may be less affected by other physicochemical parameters and can more directly indicate rice blast stress. Additionally, although rice blast typically initiates from the middle and lower parts of plants as a population-level statistical conclusion, individual plant infection is random. The presence of symptoms on a specific top 1st leaf alone cannot conclude that the top 2nd-Nth leaves of that plant are already infected.

For the top 1st leaf, the classification performance of optimal features, common features, and F760 was essentially equivalent. However, for the other three leaf positions, the classification accuracy of optimal features was greater than F760 (Figure 9). This indicates that feature selection can significantly improve monitoring performance for the top 2nd to 4th leaves. As shown in Figure 10 [Figure 10: see original paper], due to the larger leaf inclination angle and shorter leaf length of the top 1st leaf compared with middle and lower leaves during the heading stage, the top 2nd and 3rd leaves generally contribute more to canopy spectra than the top 1st leaf. The results of this study can provide reference for band selection in canopy-level rice blast SIF monitoring.

4.3 Limitations of Early RLB Monitoring Based on SIF Spectra

Although the proposed model achieved good performance for rapid early diagnosis of rice leaf blast, several limitations remain. This study used an active light source with fixed intensity to simulate sunlight, but its intensity differed from natural sunlight, resulting in SIF intensity that was not completely identical to that produced by solar irradiation. Future experiments should further investigate the intensity relationship between active light source and solar-induced fluorescence signals to correct the SIF spectra generated by active light sources. Second, during greenhouse experiments using the FluoWat leaf clip, the light intensity inside the leaf chamber was greater than outside, causing the SIF generated by measured samples to take time to reach steady state, which affected data stability. Future studies could eliminate this difference by increasing the light intensity at the pot placement area.

This study constructed leaf-scale RLB identification models using continuous wavelet spectral analysis, providing important reference for early, rapid, and in-situ diagnosis of rice blast in the field. Due to limitations in sample numbers, the influence of growth stage on monitoring accuracy at different leaf positions was not considered, although the four corresponding leaf positions across the two growth stages were at similar developmental phases. While growth stage affects leaf photosynthetic capacity, the limited number of infected samples prevented this from being a focus of discussion, which may be an important reason why the classification accuracy of models in this study needs further improvement. Future research should acquire more samples from susceptible growth stages to explore common features applicable to early RLB monitoring throughout the entire rice growth period. Additionally, due to the high infectivity of rice blast requiring rapid, non-destructive monitoring of large field areas, future work should focus on scaling up leaf-level sensitive features to the canopy level. However, canopy SIF is retrieved from upward radiance and downward irradiance spectra of rice canopies [12] and is subject to numerous environmental and internal interferences. During the scaling process, it is necessary to consider the effects of canopy structure and soil background on the sensitivity and stability of spectral features, and to develop appropriate methods for eliminating canopy structure and soil background effects.

4.4 Conclusions

Based on SIF spectra from different leaf positions, this study clarified the SIF spectral response patterns under rice blast stress, extracted RLB-sensitive SIF wavelet features using continuous wavelet analysis, and established infected leaf identification models, providing technical support for early field diagnosis of rice leaf blast. The main conclusions are as follows:

1. **Differences in identification accuracy among leaf positions were clarified.** Based on upward and downward SIF spectra, the rice blast identification model for the top 1st leaf achieved accuracies of 70% and 71%, respectively, significantly higher than those for the top 2nd, 3rd, and 4th leaves. The top 1st leaf is therefore an ideal indicator leaf for diagnosing rice leaf blast in the field.
2. **Infected leaf identification based on SIF wavelet features outperformed original SIF bands.** Through continuous wavelet transform and feature selection, the classification accuracies of optimal upward and downward features for the top 1st to 4th leaves reached 70.13%, 63.70%, 64.63%, 64.53% and 70.90%, 63.12%, 62.00%, 64.02%, respectively, all higher than the commonly used canopy monitoring feature F760.
3. **Common sensitive wavelet features applicable to multiple leaf positions were extracted.** The common features WF832,3 and WF809,3 extracted in this study achieved accuracies of 69.45%, 62.19%, 60.35%, 63.00% and 69.98%, 62.78%, 60.51%, 61.30% for the top 1st to 4th leaf positions, respectively, effectively indicating disease status across the entire plant.

References

[1] QI L, ZHANG T, ZENG J, et al. Analysis of the occurrence and control of diseases in five major rice-producing areas in China in recent years[J]. China plant protection, 2021, 41(4): 37-42, 65.

[2] TALBOT N J. On the trail of a cereal killer: Exploring the biology of *Magnaporthe grisea*[J]. Annual review of microbiology, 2003, 57: 177-202.

[3] LIU M X, ZHANG S B, HU J X, et al. Phosphorylation-guarded light-harvesting complex II contributes to broad-spectrum blast resistance in rice[J]. Proceedings of the national academy of sciences of the United States of America, 2019, 116(35): 17572-17577.

[4] KOBAYASHI T, KANDA E, KITADA K, et al. Detection of rice panicle blast with multispectral radiometer and the potential of using airborne multispectral scanners[J]. Phytopathology, 2001, 91(3): 316-323.

[5] FENG L, CHAI R Y, SUN G M, et al. Identification and classification of rice leaf blast based on multi-spectral imaging sensor[J]. Spectroscopy and spectral analysis, 2009, 29(10): 2730-2733.

[6] ZHOU L N, YU H Y, ZHANG L, et al. Rice blast prediction model based on analysis of chlorophyll fluorescence spectrum[J]. Spectroscopy and spectral analysis, 2014, 34(4): 1003-1006.

[7] ZHENG Y. Occurrence regularity and comprehensive prevention and control of rice blast[J]. Agricultural technology & equipment, 2016(4): 56-57.

[8] AN D S, WEI C B, CAO J, et al. The study of chlorophyll fluorescence characteristics on different leaf position of three sugarcane seedling cultivars[J]. Chinese journal of tropical crops, 2015, 36(11): 2019-2027.

[9] ZHANG J F, WAN L, IGATHINATHANE C, et al. Spatio-temporal heterogeneity of chlorophyll content and fluorescence response within rice (*Oryza sativa* L.) canopies under different nitrogen treatments[J]. Frontiers in plant science, 2021, 12: ID 645977.

[10] SHI Y, HUANG W J, LUO J H, et al. Detection and discrimination of pests and diseases in winter wheat based on spectral indices and kernel discriminant analysis[J]. Computers and electronics in agriculture, 2017, 141: 171-180.

[11] WANG S, MA Z H, SUN Z Y, et al. Yield loss assessment under wheat stripe rust stress based on hyperspectral remote sensing[J]. Chinese agricultural science bulletin, 2011, 27(21): 253-258.

[12] RAJI S N, SUBHASH N, RAVI V, et al. Detection of mosaic virus disease in cassava plants by sunlight-induced fluorescence imaging: A pilot study for proximal sensing[J]. International journal of remote sensing, 2015, 36(11): 2880-2897.

[13] CEN H Y, WENG H Y, YAO J N, et al. Chlorophyll fluorescence imaging uncovers photosynthetic fingerprint of Citrus huanglongbing[J]. Frontiers in plant science, 2017, 8: ID 1509.

[14] PENG J L, LI M, CHU R H, et al. Research progress of Sun-induced chlorophyll fluorescence inversion and its application in vegetation environmental stress monitoring[J]. Jiangsu agricultural sciences, 2021, 49(24): 29-40.

[15] ZHAO Y, JING X, HUANG W J, et al. Comparison of Sun-induced chlorophyll fluorescence and reflectance data on estimating severity of wheat stripe rust[J]. Spectroscopy and spectral analysis, 2019, 39(9): 2739-2745.

[16] YAN J M, JING X, ZHANG T, et al. Establishment of spectral index based on canopy SIF and PRI and its application in monitoring wheat stripe rust[J]. Journal of northwest A & F university (natural science edition), 2021, 49(12): 113-126.

[17] JIA M, ZHU J E, MA C C, et al. Difference and potential of the upward and downward Sun-induced chlorophyll fluorescence on detecting leaf nitrogen

concentration in wheat[J]. Remote sensing, 2018, 10(8): ID 1315.

[18] ZHANG Y J, HUANG W J, WANG J H, et al. Chlorophyll fluorescence sensing to detect stripe rust in wheat (*Triticum aestivum* L.) fields based on Fraunhofer lines[J]. Scientia agricultura Sinica, 2007, 40(1): 78-83.

[19] ZHANG X, YAN Y, WANG W H, et al. Application of continuous wavelet analysis to laboratory reflectance spectra for the prediction of grain amylose content in rice[J]. Acta agronomica Sinica, 2021, 47(8): 1563-1580.

[20] TIAN L, XUE B W, WANG Z Y, et al. Spectroscopic detection of rice leaf blast infection from asymptomatic to mild stages with integrated machine learning and feature selection[J]. Remote sensing of environment, 2021, 257: ID 112350.

[21] LONG W F. Control strategy of rice blast in early rice[J]. Hunan agriculture, 2007(5): ID 15.

[22] CHENG T, RIVARD B, SÁNCHEZ-AZOFEIFA G A, et al. Continuous wavelet analysis for the detection of green attack damage due to mountain pine beetle infestation[J]. Remote sensing of environment, 2010, 114(4): 899-910.

[23] CHENG T, RIAÑO D, USTIN S L. Detecting diurnal and seasonal variation in canopy water content of nut tree orchards from airborne imaging spectroscopy data using continuous wavelet analysis[J]. Remote sensing of environment, 2014, 143: 39-53.

[24] CHENG T, RIVARD B, SÁNCHEZ-AZOFEIFA A G, et al. Predicting leaf gravimetric water content from foliar reflectance across a range of plant species using continuous wavelet analysis[J]. Journal of plant physiology, 2012, 169(12): 1134-1142.

[25] LI X L, XIE C Q, HE Y, et al. Characterizing the moisture content of tea with diffuse reflectance spectroscopy using wavelet transform and multivariate analysis[J]. Sensors, 2012, 12(7): 9847-9861.

[26] SHAN Q F, ZHANG T, LI C, et al. Construction of a prediction model for mainstream cigarette smoke quality based on Fisher linear discriminant analysis method[J]. Food & machinery, 2021, 37(2): 78-84, 92.

[27] CHENG T, RIVARD B, SÁNCHEZ-AZOFEIFA A G, et al. Deriving leaf mass per area (LMA) from foliar reflectance across a variety of plant species using continuous wavelet analysis[J]. ISPRS journal of photogrammetry and remote sensing, 2014, 87: 28-38.

[28] CHENG T, RIVARD B, SÁNCHEZ-AZOFEIFA A. Spectroscopic determination of leaf water content using continuous wavelet analysis[J]. Remote sensing of environment, 2011, 115(2): 659-670.

[29] RIVARD B, FENG J, GALLIE A, et al. Continuous wavelets for the improved use of spectral libraries and hyperspectral data[J]. Remote sensing of environment, 2008, 112(6): 2850-2862.

[30] FANG M H, LIU X N. Estimation of nitrogen content in rice leaves with hyperspectral reflectance measurements using wavelet analysis[J]. Journal of applied sciences, 2010, 28(4): 387-393.

[31] MOHAMMED G H, COLOMBO R, MIDDLETON E M, et al. Remote sensing of solar-induced chlorophyll fluorescence (SIF) in vegetation: 50 years of progress[J]. Remote sensing of environment, 2019, 231: ID 111177.

[32] TIAN L, WANG Z Y, XUE B W, et al. A disease-specific spectral index tracks *Magnaporthe oryzae* infection in paddy rice from ground to space[J]. Remote sensing of environment, 2023, 285: ID 113384.

[33] XING M Y, ZHENG F C. Ultra-structural change of the rice leaf infected by *Magnaporthe grisea*[J]. Chinese journal of tropical crops, 2008, 29(1): 102-105.

[34] YI J. Study on the effects of *Magnaporthe oryzae* on photosynthesis characteristics and yield of rice plants[D]. Mianyang: Southwest University of Science and Technology, 2015.

[35] PAO Y C, CHEN T W, MOUALEU-NGANGUE D P, et al. Environmental triggers for photosynthetic protein turnover determine the optimal nitrogen distribution and partitioning in the canopy[J]. Journal of experimental botany, 2019, 70(9): 2419-2434.

[36] ADACHI S, YOSHIKAWA K, YAMANOUCHI U, et al. Fine mapping of carbon assimilation rate 8, a quantitative trait locus for flag leaf nitrogen content, stomatal conductance and photosynthesis in rice[J]. Frontiers in plant science, 2017, 8: ID 60.

[37] ZHU Y, TIAN Y C, MA J F, et al. Relationship between chlorophyll fluorescence parameters and spectral reflectance characteristics in wheat leaves[J]. Acta agronomica Sinica, 2007, 33(8): 1286-1292.

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