

Article

Digital Image Quantification of Rice Sheath Blight: Optimized Segmentation and Automatic Classification

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Abstract

Rapid and accurate phenotypic screening of rice germplasms is crucial for identifying potential sources of rice sheath blight resistance. However, visual and/or caliper-based estimations of coalescing, necrotic, diseased lesions of rice sheath blight (ShB)-infected plants are time-consuming, labor-intensive, and subject to human rater subjectivity. Here, we propose the use of RGB images and image processing techniques to quantify ShB disease progression in terms of lesion height and diseased area. To be specific, we developed a Pixel Color- and Coordinate-based K-Means Clustering (PCC-KMC) algorithm utilizing the Mahalanobis distance metric, aimed at accurately segmenting symptomatic and non-symptomatic regions within rice stem images. The performance of PCC-KMC, combined with manual classification of the segmented regions, was evaluated using Lin's concordance correlation coefficient (ρ_c) by comparing its results to visual measurements of ShB lesion height (cm) and to lesion/diseased area (cm²) measured using ImageJ. Low bias (C_b) and high precision (r) were observed for absolute lesion height ($C_b = 0.93$, $r = 0.94$) and absolute symptomatic area ($C_b = 0.98$, $r = 0.97$) studies. Furthermore, to automatically classify the segmented regions produced by the PCC-KMC algorithm, we employed a convolutional neural network (CNN). Unlike conventional CNNs that require fixed-size image inputs, our CNN is designed to take the RGB histogram of each segmented region (a 1000 by 3 representation) as input and determine whether the region corresponds to ShB infection. This design effectively handles the arbitrary sizes and irregular shapes of segmentation regions generated by PCC-KMC. Our CNN was trained based on an 85%:15% composition for the training and testing dataset from a total of 168 ShB-infected stem sample images, recording 92% accuracy and 0.21 loss. PCC-KMC-CNN also showed high accuracy and precision for the absolute lesion height ($C_b = 0.86$, $r = 0.90$) and absolute diseased area ($C_b = 0.99$, $r = 0.97$) studies, indicating that PCC-KMC combined with automatic CNN-based classification performs very effectively. These results demonstrate that the potential of our methodology to serve as an alternative to the traditional visual-based ShB disease severity assessment and can be considered to be utilized for lab-scale, high-throughput phenotyping of rice ShB.

Keywords: rice sheath blight; disease resistance screening; image-based disease phenotyping; spatio-temporal analysis; k-means clustering; convolutional neural network; Mahalanobis distance; superpixel



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1. Introduction

Rice sheath blight (ShB) is a major rice fungal disease that causes drastic reductions in the quantity and quality of rice production [1–3]. The disease has become a major rice disease in recent decades due to the intensification of the cultivation of short-statured, high-tillering and high-yielding rice varieties accompanied by increased nitrogen input [4,5]. The causal agent of ShB, *Rhizoctonia solani* Kühn AG1-IA, generates water-soaked disease lesions in the sheaths of rice leaves at the plant-water or soil interface [2]. The lesions are characterized by greenish-gray water-soaked lesions gradually maturing into grayish-white ellipsoids surrounded by dark brown to black margins on rice leaf sheaths [2,6]. The chemical management of ShB using fungicides has been effective in managing the disease in the field [1,6]. However, this approach has been increasingly discouraged due to its negative consequences for the environment and humans [7]. Furthermore, the use of host resistance is known to be the most effective and economical approach [8], preventing the persistence of the pathogen in fields that would otherwise lead to continuous ShB outbreaks [1]. Nevertheless, to date, rice germplasm with complete resistance to ShB has not yet been identified [9–12]. Hence, the screening of rice germplasm in search of sources of ShB resistance is crucial. [13].

Currently, there are numerous approaches to screen for ShB resistance in field and controlled conditions [9,13,14]. For example, the microchamber method provides a conducive environment for optimal ShB development and allows minimal environmental effects [9]. Quantification of ShB resistance is generally achieved using visual estimations of the diseased area relative to the whole plant or ruler-based measurements of the vertical distance in which the disease lesions have progressed (lesion length) [13–16]. The limitation of this visual-based disease phenotyping method is that it is time-consuming and vulnerable to subjectivity and inaccuracy of the rater, specifically when the screening is conducted on large scales (hundreds of plants) [9,13,17–19]. Moreover, inconsistencies in evaluating and scoring ShB disease reactions in screening experiments arise due to the subjectivity of the variety of ShB disease scoring systems based on visual estimations. In addition, disease scoring or measurement methods, including measurement of lesion length (in centimeters), disease scales (0–9), percentages of disease lesions based on lesion length and leaf position, differ depending on artificial inoculation methods [13]. Precise phenotypic data are crucial in screening experiments [20], particularly for the screening of resistance to polygenic diseases, due to the widely varying susceptibility profiles between different rice cultivars [1,21,22]. Hence, utilizing repeatable and less subjective approaches, including an image-based disease quantification method, will capture minor differences in terms of resistance reactions and may provide reliable and precise phenotypic data, which will help find stronger and more robust germplasm in resistance breeding programs.

The use of imaging systems has shown great promise as a tool for quantifying plant diseases, including red-green-blue (RGB), multispectral, and hyperspectral images [23–25]. These technologies, along with convolutional neural networks, show promise in assessing disease severity and can reduce human errors caused by visual assessments [17,26–28]. Some image processing platforms are currently available for assessing the severity of plant disease, including Assess (v. 2.0) [29], SigmaPlot (v. 12.5) [30], and ImageJ (v. 1.53) [31]. Furthermore, numerous studies on the detection of rice diseases have been documented with RGB imagery [32–34]. However, none has yet been developed specifically targeted for ShB phenotyping. Moreover, a crucial point that must be addressed for new plant disease assessment methods is their ability to generate accurate measurements by validation of the measurement of the proposed method. ‘Accuracy’ refers to the closeness of the measured value to the actual value, while ‘precision’ refers to the variability of the measurement [17,35]. Using these parameters, validation can be achieved by comparing the measurements of the

new method with actual values or ‘gold standard’, such as visual-based measurements or pre-existing, image-based measurements (ImageJ) [17].

In general, plant disease detection using digital image processing consists of three major steps: (i) pre-processing (background and noise removal), (ii) segmentation, and (iii) feature extraction and identification of the object of interest [23,36,37]. Among these different image processing steps, image segmentation is a crucial process that delineates and groups different regions of an image. One of the most commonly used image segmentation algorithms for plant disease detection has been K-Means Clustering (KMC) [38–41]. Although KMC segments RGB images into ‘k’ groups based on the proximity of the RGB color space, it is limited in terms of spatial proximity of pixels, which becomes a significant issue in detecting the coalescing and necrotic nature of ShB lesions in ShB-infected stems that do not appear in a sporadic random pattern, suggesting that this segmentation method may not reflect the distinct spatial patterns of water-soaked ShB infection.

Conventional rice ShB phenotyping typically relies on the ordinal scale IRRI-standard 0–9 or 0–10 based solely on the maximum height of the injury. However, this scale does not incorporate the area of the injury, provides limited resolution to detect subtle progression in early stages of the disease, and requires substantial human labor while remaining highly dependent on visual raters and, therefore, prone to subjectivity. In our study, we developed an objective approach to score ShB using the standardized laboratory micro-chamber screening method developed to screen rice seedlings under controlled growth chamber or greenhouse conditions. This method is known to expedite the selection process for searching for sources of ShB resistance. To address these limitations, we introduce a digital image processing and machine learning-based framework that enables an objective, automated evaluation of ShB severity. The results demonstrate that the proposed pipeline can serve as a promising tool for high-throughput phenotyping of rice ShB. The methodological innovations and contributions of this work are summarized as follows:

- The proposed method quantifies both the lesion area and the maximum height and, once sample images are provided, can rapidly and automatically evaluate the severity of the disease without manual intervention. This enables large-scale phenotyping with significantly reduced labor requirements.
- Methodologically, the framework consists of two major components:
 1. Segmentation of lesion and healthy regions using the proposed Pixel Color- and Coordinate-based K-Means Clustering algorithm (PCC-KMC) utilizing the Mahalanobis metric;
 2. Automated classification using a pre-trained binary Convolutional Neural Network (CNN) classifier that determines whether each segmented region corresponds to an ShB lesion.
- In the segmentation step, traditional unsupervised color-based k-means clustering fails to isolate local lesions because it groups spatially scattered pixels with similar color values. By incorporating the Mahalanobis distance into the clustering, the proposed PCC-KMC method enforces spatial locality and color similarity simultaneously, allowing accurate extraction of local ShB lesions.
- Instead of relying on manual visual judgment to determine whether each segmented region is diseased, we employ a CNN-based classifier for automatic classification.
- Conventional CNNs require fixed-size rectangular image inputs and, therefore, cannot directly handle the irregular shapes and variable sizes of segmented lesion patches. To overcome this, we extract RGB histograms from each segmented region and use them as compact CNN inputs, leveraging the fact that the color distribution differs between the lesion and the healthy tissue.

- Because RGB histograms can be normalized to a fixed dimension regardless of the shape or size of the region, they are ideally suited as CNN input for robust classification.

Using the developed PCC-KMC-CNN pipeline, one can further demonstrate its utility by distinguishing rice species with different levels of resistance to ShB.

2. Related Works

2.1. Traditional Phenotyping of Rice Sheath Blight

Rice ShB phenotyping has historically relied on visual evaluation of the height of ShB lesion. Classical studies established protocols for inoculation, field evaluation, and manual scoring of lesion spread along the culm [9,42–47]. These approaches provide practical field-level guidelines, but inherently depend on rater experience, suffer from scale sparsity in early disease stages, and require substantial labor for large-scale screening. Despite their importance, traditional phenotyping methods do not quantify the precise area of the lesion and offer limited reproducibility, motivating the need for automated digital pipelines [48,49].

2.2. Genetic, Genomic, and QTL Studies and ShB Quantification

Significant efforts have been made to investigate the genetic basis of ShB resistance through Quantitative Trait Locus (QTL) mapping, Genome-Wide Association Studies (GWAS), and genomic analyzes. Early foundational studies reported major QTLs such as *qSBR11-1*, *qSB9-2*, *qSB12-1*, and others in diverse rice populations [11,50–53]. Subsequent research refined the mapping resolution, identifying loci such as *qSB-11LE* and additional candidate genomic regions associated with partial resistance [54–58]. These findings were complemented by analyzes of plant architectural traits correlated with disease severity [14,59] and molecular insights into host-pathogen interaction mechanisms [60–62]. Reviews summarizing global progress in ShB resistance have emphasized the importance of accurate phenotyping as input for genetic studies [63–67]. However, most genomic studies continue to rely on manual lesion height scoring, which limits performance and introduces unavoidable subjectivity.

2.3. Digital Imaging and RGB-Based Disease Quantification

Recent advances in computer vision have enabled RGB-image-based analysis of foliar diseases. For example, Lee et al. developed contour-based stomata detection algorithm and deep-learning-supported quantification pipelines for corn tar spot using RGB imagery [68,69]. These methods highlight the potential for object-based stomata lesion detection, but target diseases with these structures rather than continuous lesion gradients, which is typical for ShB. In the context of rice, unmanned aerial system imaging and high-resolution RGB data have been utilized for canopy-level ShB detection [70], but these approaches do not resolve the geometry of the culm-level lesion or provide pixel-wise lesion segmentation. No previous RGB-based work has integrated spatial pixel coordinates and color features within a unified clustering scheme nor incorporated cluster-level CNN classification for automatic annotation as proposed in the PCC-KMC-CNN pipeline.

2.4. Hyperspectral and Multispectral Disease Detection Approaches

Beyond using RGB image data, spectral signatures have also been used to detect ShB symptoms and pre-symptomatic stress signals. Conrad et al. applied machine learning to spectral reflectance profiles to detect ShB-infected lesions of the early phase [71], while Zhang et al. implemented in-situ hyperspectral imaging for the detection of lesion directly on rice stalks [72]. Although powerful, hyperspectral systems require expensive specialized hardware and are less feasible for routine small-scale laboratory breeding programs. In

contrast, our RGB-based PCC-KMC-CNN framework focuses on utilizing low-cost imaging while still offering pixel-level lesion masks and automated classification.

2.5. Summary of Gaps in the Literature

In general, the above literature review reveals few limitations, such as (1) the dominant reliance on manual lesion height scoring, (2) the lack of accurate lesion segmentation for ShB and automated classification algorithms, and (3) the high barriers to scalability. To address these gaps, we propose the PCC-KMC-CNN pipeline, which (1) is capable of effectively segmenting ShB-infected regions by integrating pixel color and coordinate features simultaneously, and (2) automatically labels lesion clusters through CNN-based histogram classification. This enables quantifying both both maximum lesion height and the proportional lesion area, offering a fully automated and high-throughput phenotyping solution for rice ShB.

3. Materials and Methods

3.1. Preparation of ShB-Infected Plant Samples

The rice cultivars/accessions were obtained from the Genetic Stocks *Oryza* Collection, USDA-ARS, Dale Bumpers National Rice Research Center, Stuttgart, Arkansas [73]. Ten rice accessions were randomly selected from the Rice Diversity Panel 1 (RDP1) (Table 1), a collection of purified, homozygous *Oryza sativa* L. accessions encompassing land races and elite rice cultivars [73].

Table 1. List of ten RDP1 rice accessions and the number of RGB images used for testing PCC-KMC-CNN.

GSOR ID [†]	Name	Original Providing Country	Region	Subpopulation (Structure) [‡]	Subpopulation (PCA) [#]	Number of RGB Images
301065	IR 8	Philippines	Southeast Asia	Indica	Indica	20
301091	LAC 23	Liberia	Africa	Tropical japonica	Tropical japonica	12
301093	Lemont	United States	North America	Tropical japonica	Tropical japonica	26
301123	Rathuwee	Sri Lanka	South Asia	Indica	Indica	14
301332	Cenit	Argentina	South America	Tropical japonica	Tropical japonica	16
301341	ARC 10376	India	South Asia	Aus	Aus	14
301351	Rikuto Norin 21	Japan	East Asia	Temperate japonica	ADMIX *	14
301358	Santhi Sufaid	Pakistan	South Asia	Aus	Aus	26
301402	LaGrue	United States	North America	Tropical japonica	Tropical japonica	4
301406	Jasmine85	Philippines	Southeast Asia	Indica	Indica	22

[†] GSOR: Genetic Stocks-*Oryza* collection identification number. * ADMIX is a mixture of more than one subpopulation. [‡] Subpopulation identified by STRUCTURE analysis using 36 SSRs [74]. [#] Subpopulation identified by PCA using 36,901 high-quality SNPs [75].

De-husked rice seeds of the 10 cultivars, 301065 (IR 8), 301091 (LAC 23), 301093 (Lemont), 301123 (Rathuwee), 301332 (Cenit), 301341 (ARC 10376), 301351 (Rikuto Norin 21), 301358 (Santhi Sufaid), 301402 (LaGrue), and 301406 (Jasmine 85), were surface-sterilized with 10% Chlorox solution (The Clorox Company, Oakland, CA, USA), germinated on 1/2 Murashige and Skoog (1/2 MS) medium (Murashige and Skoog Basal Medium; Sigma-Aldrich, St. Louis, MO, USA), and then transplanted into plastic pots (15 cm in diameter) filled with ProMix BXTM soil (Premier Tech Horticulture, Rivière-du-Loup, QC, Canada) in Figure 1. The seedlings were grown in a growth chamber (26 °C on day and 20 °C at night, 12 h light/dark cycle, 80% relative humidity) until they were ready for inoculation (Figure 1).

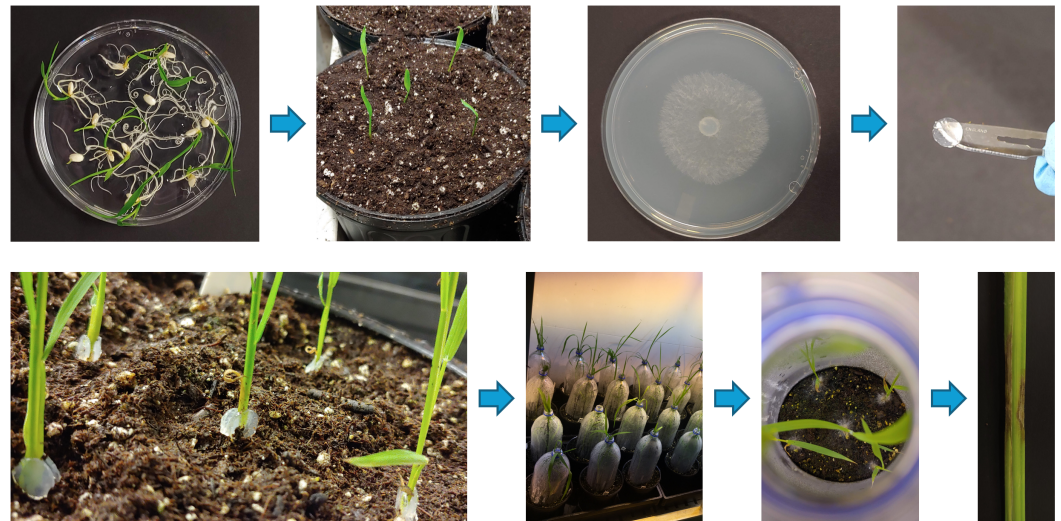


Figure 1. Pictorial diagram depicting the steps in preparing rice ShB-infected rice stem samples for RGB imaging.

Rice seedlings with four to five leaves were then inoculated with the ShB pathogen *R. solani* (isolate B2 provided by Dr. Jim Correll, University of Arkansas, USA). Circular agar blocks (5 mm diameter) were excised from the border of an actively growing 3-day-old culture on potato dextrose agar (PDA). The agar blocks were attached on both sides of the stem base of each seedling (two agar blocks per seedling). The microchamber method [9] was used, and inoculated seedlings were placed in growth chambers at 28–30 °C with a relative humidity of ~90% to provide optimum conditions for ShB development. At 7 to 10 days after inoculation, the distance between the point of pathogen inoculation (base of the rice plant) and the margin of the lesion farthest from the base of the stem was measured using a caliper to reflect the severity of the disease [2,6,9,47]. After collecting visual-based measurements of ShB progression, the stem of the rice plant was excised to acquire RGB digital images.

3.2. Acquisition of RGB Digital Images of ShB-Infected Rice Stems

In this study, all digital RGB images of rice stems were acquired under controlled laboratory conditions using HP Scanjet G4050 flatbed scanner (HP Inc., Palo Alto, CA, USA). To minimize background noise and facilitate accurate cropping of the region of interest, the stem samples were placed on a monochromatic matte black background prior to scanning. Images were acquired at a fixed resolution of 600 pixels per inch (ppi) and saved in PNG format, ensuring consistent lighting, viewing angle, and imaging geometry across all samples. Under these controlled conditions, variations in illumination, perspective, and resolution are negligible, thereby reducing pre-processing challenges associated with such variability. All subsequent digital image processing was performed using the Image Processing Toolbox in (MathWorks Inc., Natick, MA, USA) [76]. However, for field-scale applications, substantial variations in lighting, shading, camera angle, and image sharpness can significantly affect segmentation and classification performance. In such scenarios, additional pre-processing steps—such as illumination correction, histogram normalization, color calibration using reference cards, and geometric alignment—would be required to maintain robustness.

Specifically, we pre-process an input image to stabilize illumination and suppress residual noise. First, we apply Gaussian smoothing to each RGB channel using MATLAB's `imgaussfilt` with a standard deviation of 5. This step attenuates salt-and-pepper-like speckles and other high-frequency artifacts while preserving the low-frequency structure

that distinguishes healthy from diseased tissue. Next, we perform flat-field correction with MATLAB's `imflatfield` with a radius of 50 pixels to avoid bias from background pixels. Flat-fielding compensates for uneven illumination and vignetting, effectively normalizing shading across the region of interest and enhancing the contrast between healthy and diseased regions, which improves the reliability of downstream color-based screening and segmentation.

3.3. Image Analyses of ShB-Infected Rice Stems

3.3.1. Pre-Processing of Acquired RGB Images

All of the acquired RGB images were pre-processed to remove the background and isolate the region of interest (rice stem image) (Figure 2a).

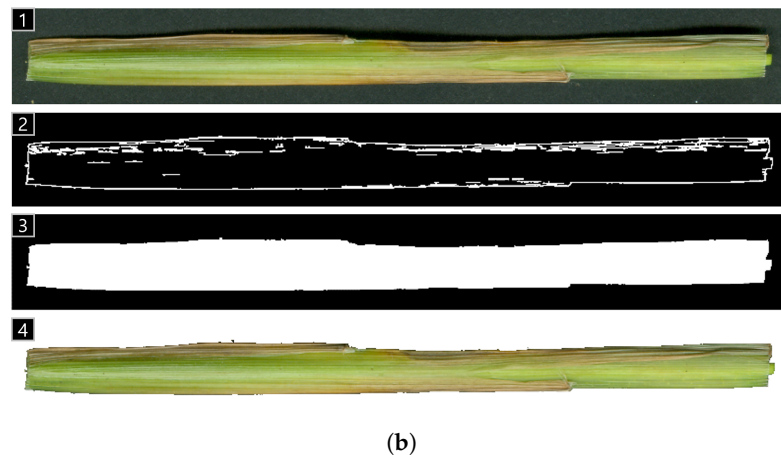
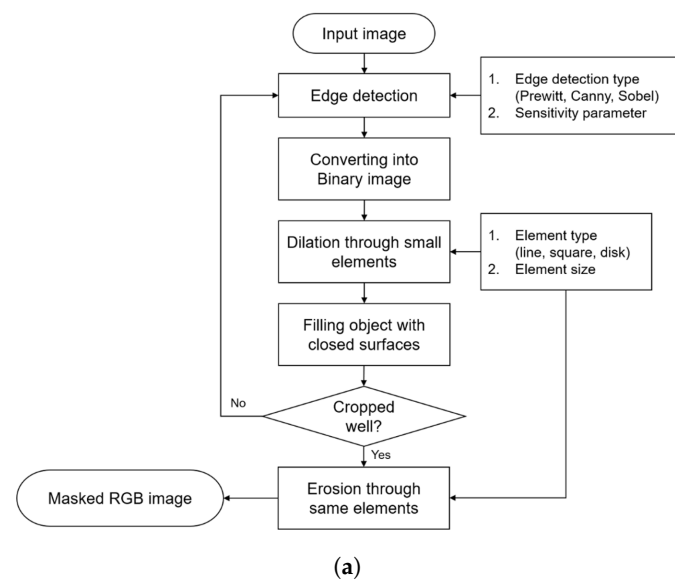


Figure 2. Pre-processing steps of RGB images to isolate the plant sample of interest. (a), Schematic diagram of the background removal steps employed in this study. (b-1) Input RGB digital image of the stem of a sheath blight-infected rice plant (cultivar Jasmine 85). (b-2) Binary images after using the edge detection with dilation. (b-3), Binary images after filling and erosion. (b-4), the masked RGB digital image after (b-2,b-3) were completed.

Upon isolation of the stem image, green-dominant pixels (green, non-symptomatic tissue) were extracted through initial screening using the following lenient threshold criterion:

$$I_G(r, c) > I_R(r, c), \quad I_G(r, c) > I_B(r, c), \quad (1)$$

where I_R , I_G , and I_B represent red, green, and blue values of a given input RGB digital image, respectively, whereas r and c indicate row and column pixel index, respectively. Pixels that met the above condition were labeled as 1; otherwise, they were labeled as 0, thereby generating two binary images/masks to decompose the input RGB into green tissue (non-symptomatic tissue) and disease lesions (symptomatic tissue). For instance, the acquired RGB image of the Jasmine 85 stem (Figure 2(b-1)) was pre-processed using two key parameters associated with the performance of the background remover: (i) the edge detection type with a sensitivity parameter (Figure 2(b-2)) and (ii) the element type and size for dilation and erosion (Figure 2(b-3)). Consequently, successful isolation of the rice stem from the background was achieved (Figure 2(b-4)) and the well-masked RGB image of Jasmine 85 was then subjected to color thresholding that decomposed the rice stem into regions without ShB disease symptoms (green regions) and with disease symptoms (necrotic lesions) (Figure 3A).

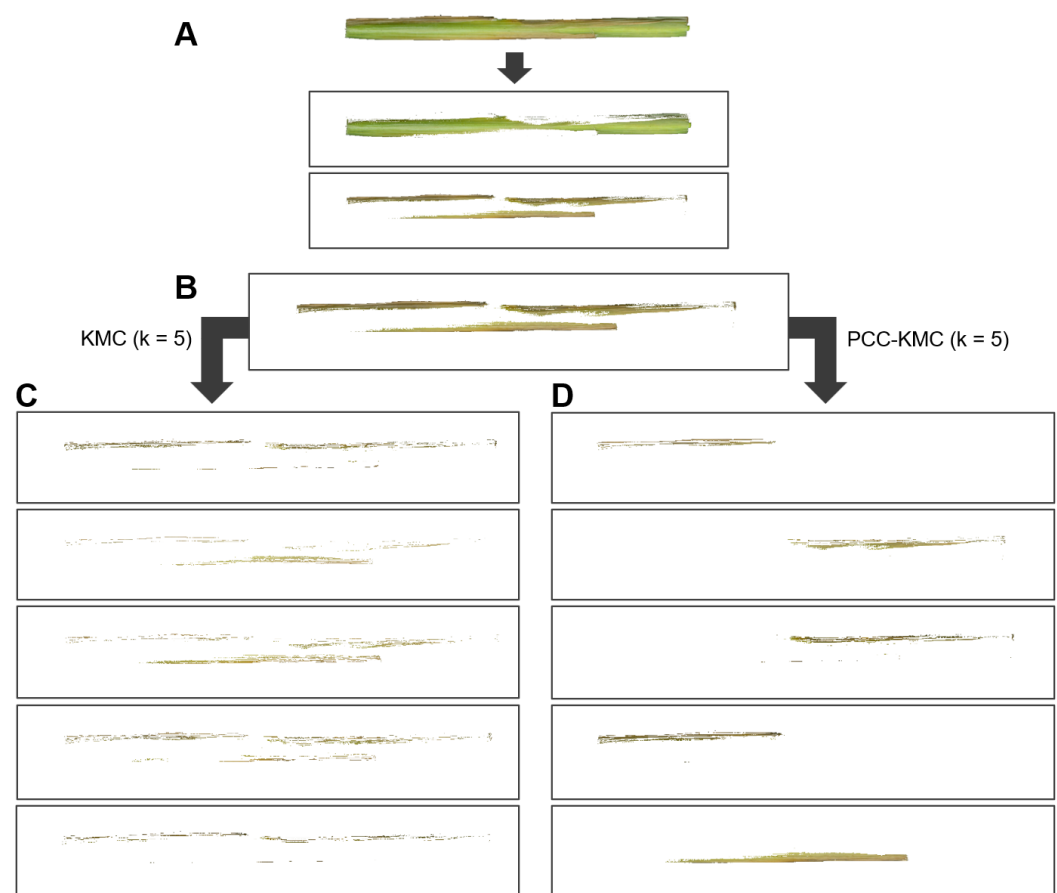


Figure 3. Image segmentation of the stem of an ShB-infected rice plant (cultivar Jasmine 85). (A), Segmentation of the ShB-infected Jasmine 85 sample using a simple thresholding method to partition plant regions without disease symptoms (green pixels) from those with disease symptoms. (B), Working principle for image segmentation used in KMC and PCC-KMC algorithms. Pattern of pixel clustering using (C), KMC and (D), PCC-KMC.

3.3.2. Segmentation of RGB Images Through PCC-KMC

Since ShB gradually progresses towards the rice canopy and infects leaf blades, leaving disease lesions along its progression path (vertical and horizontal spread) [6], we developed an optimal segmentation method that accounts for spatial proximity as well as color similarity while clustering. We developed a pixel color- and coordinate-based k-means clustering algorithm (PCC-KMC) operating over 5-dimensional space composed of RGB color values and row and column pixel index, i.e., red, green, blue, row, and column, wherein the measure of the distance between two data points directly affects the result of k-means clustering. Moreover, instead of utilizing the standard Euclidean distance, we utilized the Mahalanobis metric, which can properly account for the scaling difference in each axis incorporated into the covariance matrix [77] in order to define the closeness between a pair of data points over the 5-dimensional space. That is, for arbitrary two data points $\mathbf{x}_1 = \{R_1, G_1, B_1, r_1, c_1\}^T$ and $\mathbf{x}_2 = \{R_2, G_2, B_2, r_2, c_2\}^T$, one can evaluate the Mahalanobis distance between the data points as

$$d_M(\mathbf{x}_1, \mathbf{x}_2) = \sqrt{(\mathbf{x}_1 - \mathbf{x}_2)^T \cdot \bar{\mathbf{C}}^{-1} \cdot (\mathbf{x}_1 - \mathbf{x}_2)} \quad (2)$$

where $\bar{\mathbf{C}}$ denotes covariance matrix. As a result, PCC-KMC allows clustering a set of data points that are statistically proximal. Given a set of L number of observations $(\mathbf{y}_1, \mathbf{y}_2, \dots, \mathbf{y}_L)$, each observation includes RGB color values at an r th and c th pixel, i.e., $\mathbf{y}_i = \{R_i, G_i, B_i, r_i, c_i\}$, where L is equal to the total number of pixels of the possibly symptomatic regions which were labeled by 0 from the initial screening. Furthermore, PCC-KMC partitions L observations into a given number $K (\leq L)$ sets, denoted as $\mathbf{S} = \{S_1, S_1, \dots, S_K\}$, in such a way as to minimize the total sum of the Mahalanobis distances between observations and centroids of the clusters, such as

$$\arg \min_{\mathbf{S}} \sum_{i=1}^K \sum_{\mathbf{y}_j \in S_i} \sqrt{(\mathbf{y}_1 - \mathbf{y}_2)^T \cdot \bar{\mathbf{C}}^{-1} \cdot (\mathbf{y}_1 - \mathbf{y}_2)} \quad (3)$$

where μ_i is the mean of points S_i .

To examine the performance of PCC-KMC in accurately extracting disease progression of ShB in Jasmine 85, segmentation patterns of PCC-KMC and KMC ($k = 5$) were compared (Figure 3B). KMC generated clusters that contained pixels that were similar in terms of color (RGB values) regardless of the location of the pixels in the image (Figure 3C). On the contrary, PCC-KMC clustered regions in terms of color similarity and spatial proximity, as reflected by each cluster where stem regions consisting of pixels of similar colors that were also in close proximity were grouped together (Figure 3D).

Note that when running the PCC-KMC algorithm, the only tunable parameter is the number of K-means iterations used to refine the initial clustering. We set this value to 10, which provided stable and well-converged segmentation results. In our implementation, the initial cluster means are selected by randomly sampling K points from the dataset. The algorithm then performs 10 iterations, each consisting of assigning points to the nearest cluster center and recomputing updated centers. Through these iterations, the within-cluster variance is progressively reduced, leading to more stable and coherent segmentation outcomes.

3.3.3. Testing the Performance of PCC-KMC to Other Existing Methods

The performance of PCC-KMC was further evaluated by comparing it to other segmentation approaches using a different ShB-infected sample image (301056): (i) k-means clustering over RGB color space with the Euclidean metric (KMC), (ii) k-means clustering over 5-dimensional color and pixel space with the Mahalanobis metric (PCC-KMC),

(iii) mean-shift clustering [78] over 5-dimensional color and pixel space with the Mahalanobis metric (PCC-MS), and (iv) simple linear iterative clustering (SLIC) (Figure 4a). The number of clusters was pre-set as 20 and bandwidth as 1 since the use of KMC, PCC-KMC and SLIC requires the number of clusters, whereas PCC-MS requires the bandwidth information or window size. Note that SLIC was included solely as a baseline method for benchmarking the segmentation performance of our PCC-KMC algorithm. As a well-established superpixel segmentation technique that incorporates both spatial proximity and color similarity, SLIC serves as an appropriate reference for assessing how effectively PCC-KMC isolates ShB lesions.

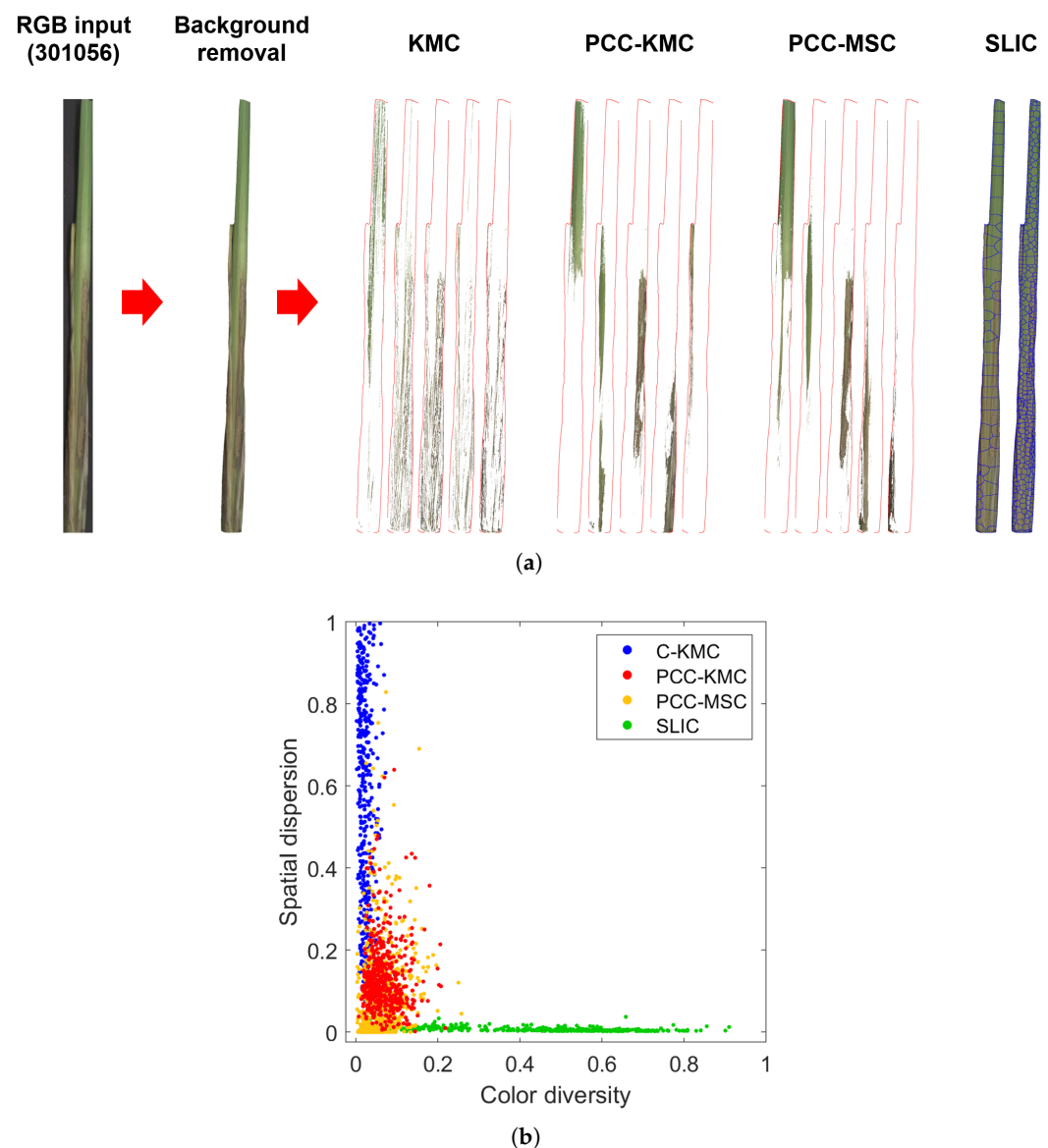


Figure 4. Comparison of the performance of various image segmentation methods. (a), Illustration of representative clusters produced by KMC, PCC-KMC, PCC-MS, and SLIC for a sample stem (301056) RGB image ($k = 20$). Here, the red arrows indicate the processing pipeline from the raw RGB image to background removal, followed by clustering using the different segmentation methods. (b), Clusters generated by the four different segmentation methods distributed based on color diversity and spatial dispersion axes for a total of 26 sample stem images.

An overall comparison of the performance of each segmentation method was performed to illustrate the extent of color diversity versus spatial dispersion for all clusters

obtained from segmenting 26 selected ShB-infected RGB images (Figure 4b). Here, for each cluster, the color diversity is defined by

$$\text{color diversity} = \text{tr}(\bar{\mathbf{D}}_c) / \text{tr}(\bar{\mathbf{D}}_t) \quad (4)$$

where $\text{tr}(\cdot)$ denotes the trace that evaluates the sum of diagonal entries of a matrix and $\bar{\mathbf{D}}_c$ and $\bar{\mathbf{D}}_t$ are 3 by 3 diagonal matrices whose elements correspond to eigenvalues of covariance matrices of RGB color values of data points in a cluster. The smaller the color diversity, the purer the color quality a cluster has. Similarly, the spatial dispersion can be defined by

$$\text{spatial dispersion} = \text{tr}(\bar{\mathbf{E}}_c) / \text{tr}(\bar{\mathbf{E}}_t) \quad (5)$$

where $\bar{\mathbf{E}}_c$ and $\bar{\mathbf{E}}_t$ are 2 by 2 diagonal matrices whose elements are eigenvalues of covariance matrices of row and column pixel index of data points in a cluster. The larger the spatial dispersion becomes, the spatially less-local pixels comprise a cluster.

3.3.4. Convolutional Neural Network-Based Automatic ShB Symptom Classification

Each of the clusters generated after being processed by PCC-KMC needs to be classified into ShB symptomatic or non-symptomatic cluster. In order to automate this classification step, a convolutional neural network (CNN) was employed. A total of 142 ShB-infected rice stem images were PCC-KMC processed, wherein each image was segmented into 20 clusters. A total of 2840 clusters were classified into one of four classes, namely (i) ShB lesion, (ii) gradation (symptomatic region surrounding ShB lesion), (iii) green tissue (non-symptomatic region) and (4) edge (border of plant samples, resulting from imperfect background removal). RGB color histograms of all images were also included as input dataset to train the CNN classifier. However, due to inhomogeneity of images in terms of brightness, contrast, gamma and so forth, we normalized the RGB color histogram of each cluster in a range from 0 to 1 (double format) in which the minimum and the maximum values of the RGB color histogram before normalization correspond to 0 and 1, respectively. As a result, the resulting aggregate input dataset was stored in a 3-dimensional array sized by $1000 \times 3 \times 2840$. Default functions, such as `trainNetwork` and `convolution2dLayer`, provided by MATLAB [76], were utilized to train the CNN classifier and design a 2-dimensional convolutional layer. For the optimal performance, we applied the 85:15 ratio rule between training and testing sets and manually classified the clusters of the training set to obtain ground truth. The detailed parameters are listed in Table 2.

Table 2. Parameters to build a CNN architecture.

Parameter	Value
filter kernel	uniform
filter size	10×3
initial learning rate	0.001
training option	SGDM with 0.9 momentum
maximum epochs	30
minimum batch size	100

Our CNN classifier consists of a single two-dimensional convolution layer, a ReLU layer, a fully connected layer sized by 4, and a softmax layer. The 2-dimensional convolution layer employs a uniform filter sized by 10×3 . The CNN architecture was trained with the stochastic gradient descent with momentum (SGDM) with momentum 0.9, initial learning

rate 0.001, maximum epochs 30 (an epoch is a full pass through the entire dataset), and minimum batch size 100.

Figure 5A,B present the training progress plot, depicting accuracy and loss versus epoch, respectively. The final accuracy and loss were recorded as 92.00% and 0.2117, respectively.

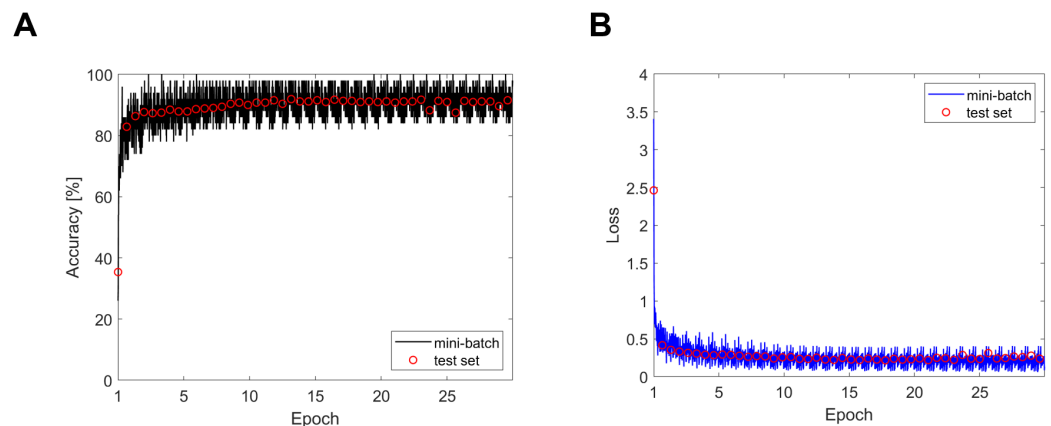


Figure 5. Training progress plot of a convolutional neural network. (A), Mini-batch accuracy [%] versus Epoch for training and test sets (black solid line and red circle, respectively). (B), Mini-batch loss versus Epoch for training and test sets (blue solid line and red circle, respectively).

3.3.5. Post-Processing to Quantify Disease Severity

For each symptomatic binary mask after CNN classification, we performed post-processing to quantify the disease severity in terms of absolute and proportional lesion height and area. The absolute lesion area was calculated by scaling the total number of pixels at which a binary value is equal to unity. In our experiments, since the real and pixel sizes of a scanned image were 21.77 cm $\times$ 31.29 cm and (5135 $\times$ 7383), respectively, the absolute area represented by each pixel was calculated as $(21.77 \times 31.29) / (5135 \times 7383) = 1.796810^{-5}$ cm² and the absolute length represented by one side of each pixel was calculated as $\sqrt{1.796810^{-5}} = 4.238910^{-3}$ cm. The total number of pixels from a binary mask was counted using the MATLAB `bwarea` function. In addition, a proportion of the diseased area was obtained by dividing the absolute symptomatic area with the absolute area of the stem. Similarly, the absolute height of the lesion was calculated by scaling the number of pixels from the base of the stem to the lesion of the disease farthest from the base. For cases where noises or incorrectly classified symptomatic regions were distributed over higher parts of the stems, the measure of lesion height was significantly altered, even if the portion of the artifacts is extremely small compared to the total region of the disease. To avoid these situations, we calculated a cumulative distribution function (CDF) of the symptomatic area with respect to the height of the stem. Then, for a given height, the CDF returned a value ranging from 0 to 1, representing the relative portion of the symptomatic region. We visualized this CDF using a bar graph, which is opaque up to 0.9 of the CDF and transparent from 0.9 to 1 of the CDF with the extent of the CDF value in Figure 6. As such, we can compare the performance of image processing-based disease quantification to visual rating. As a note, the reference (visual rating) is the method by which a human rater visually measured the lesion height on the original RGB image of the ShB-infected stem using MATLAB.

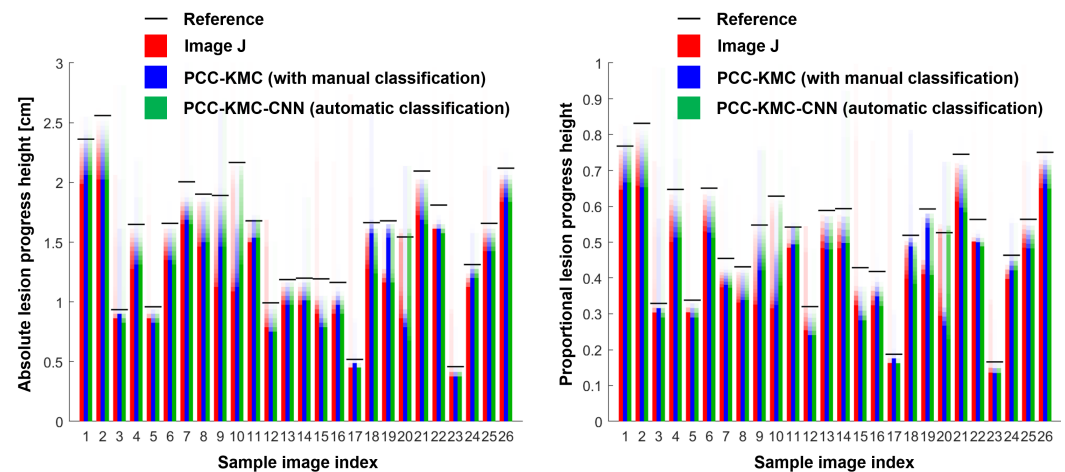


Figure 6. Comparison of lesion height and diseased area measurements obtained from manual annotation, ImageJ, PCC-KMC and PCC-KMC-CNN. Bar graph depicting absolute and proportional lesion heights of 26 ShB-infected rice culm images. Each bar is visualized by being transparent where the CDF value of the detected diseased region starts from 0.9, in which the extent of the transparency depends on the CDF value.

3.4. Evaluation of the Accuracy of PCC-KMC and PCC-KMC-CNN

An algorithmic overview of the present PCC-KMC-CNN with the Mahalanobis distance metric is depicted in Figure 7.

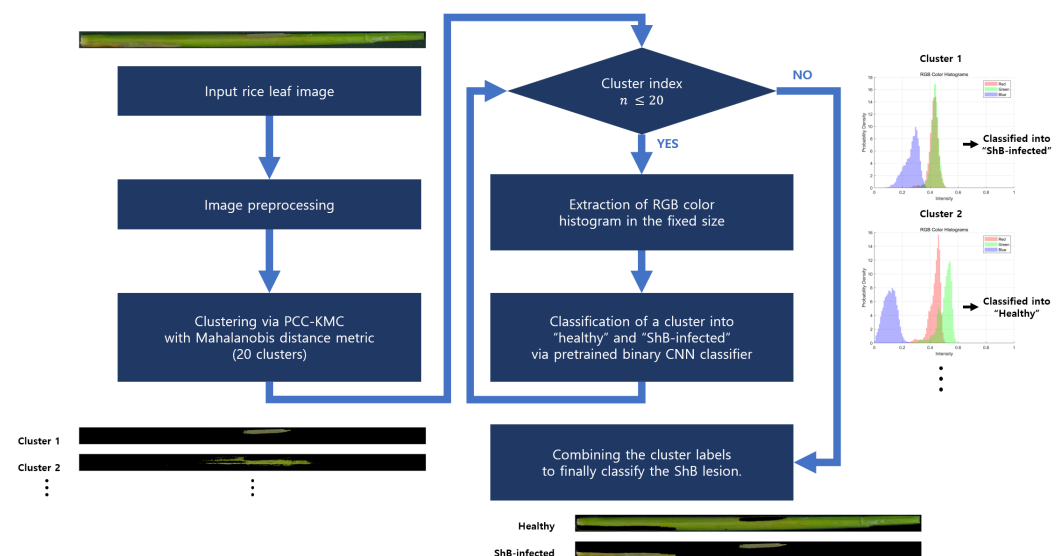


Figure 7. Algorithmic flow of the developed PCC-KMC-CNN pipeline.

To validate the results obtained by the PCC-KMC algorithm, we measured the lesion height of ShB progress on original RGB images using MATLAB to serve as the reference of lesion height measurement. As for the reference measurement for the symptomatic disease area, we utilized the color thresholding feature in ImageJ [31], wherein we obtained a symptomatic binary mask, which was then post-processed in MATLAB to extract lesion height and area. ImageJ has been widely used as a gold standard in image-based plant disease quantification [17,79], hence serving as the actual value in comparing the symptomatic diseased area generated by PCC-KMC and PCC-KMC-CNN. Moreover, color thresholding was performed in LAB color space with ad hoc thresholding values for every sample image. Finally, using 26 sample images that were not a part of the training set in the CNN classifier, we compared results (lesion length and the area of the diseased region) generated by visual

assessment, ImageJ, PCC-KMC with manual labeling, and the PCC-KMC-CNN classifier. The accuracy and precision of the measurements by PCC-KMC and PCC-KMC-CNN were tested using the Lin's concordance correlation coefficient [80].

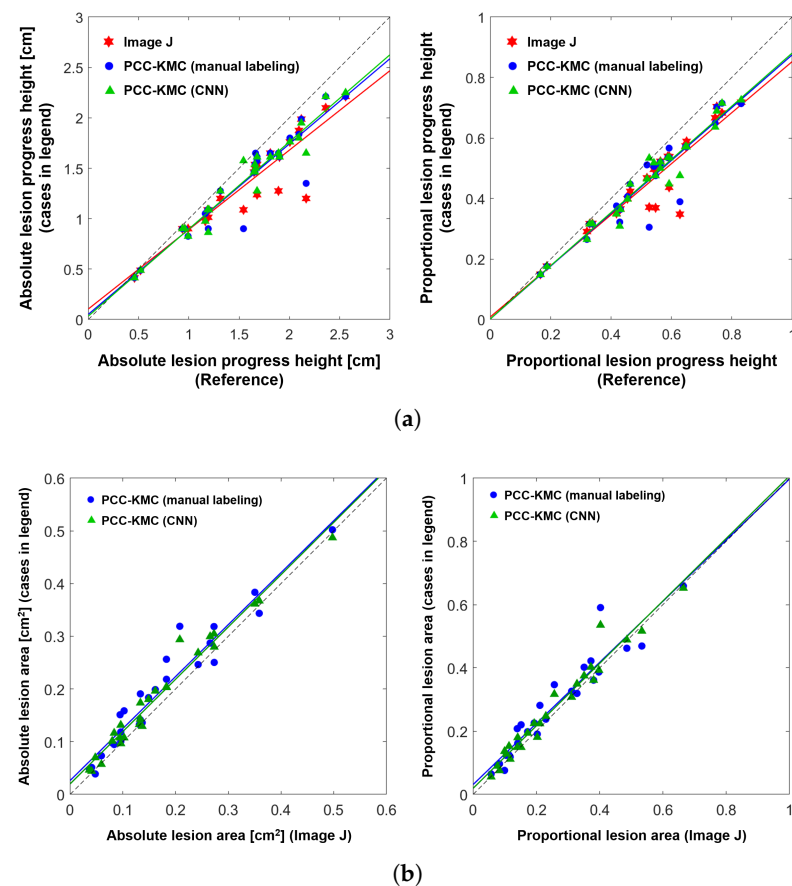


Figure 8. Comparison of lesion height and diseased area measurements obtained from manual annotation, ImageJ, PCC-KMC and PCC-KMC-CNN. (a) Agreement between lesion heights obtained using manual annotation and those obtained using ImageJ, PCC-KMC and PCC-KMC-CNN. (b) Agreement between diseased area obtained using ImageJ, PCC-KMC and PCC-KMC-CNN. Note that the blue and green solid lines represent the regression lines for PCC-KMC with manual labeling and PCC-KMC-CNN, respectively, whereas the black dashed line denotes the reference line indicating exact correlation.

4. Results

4.1. Performance of PCC-KMC

Figure 4a compares the segmentation results by KMC, PCC-KMC, PCC-MSL and SLIC. KMC tends to produce clusters in which pixels in each cluster possess higher color correlations and fewer spatial correlations. In contrast, both PCC-KMC and PCC-MSL effectively separated symptomatic and non-symptomatic clusters. In the case of SLIC, the resulting clustered regions were spatially over-localized, while RGB color values were less correlated. Although performance improved by increasing the number of clusters ($N_k = 2000$, as seen in the right stem image under SLIC), it was not an efficient method to train a convolutional neural network, as one had to label too many clusters in each image. PCC-KMC and PCC-MSL produced clusters that had the lowest spatial dispersion and color diversity, which coincided with the result shown in Figure 4a. Collectively, since ShB symptomatic regions were often spatially localized with different colors, the PCC-KMC and PCC-MSL segmentation methods were appropriate for our purpose to correctly extract features from symptomatic regions (Figure 4b). Moreover, KMC generated clusters that

only had the smaller color diversity but the larger spatial diversity and the opposite trend was observed for SLIC. Finally, KMC and SLIC were not suitable for accurate extraction of features or quantification of plant disease, as segmented regions did not represent ShB symptomatic regions.

4.2. Accuracy of ShB Severity Measurements Obtained from Visual Manual Annotation, ImageJ and PCC-KMC and PCC-KMC-CNN

For all sample images, we observed that the reference results were lower and upper bounded by the CDF values 0.89 and 0.97, respectively (the average CDF value was around 0.93). This result suggests that visual rating and image processing-based disease estimates were within a close range of values (Figure 8).

Agreement (ρ_c) of absolute lesion height (cm) among different methods to visual measurement was highest for PCC-KMC ($\rho_c = 0.87$), followed by ImageJ ($\rho_c = 0.83$) and PCC-KMC-CNN ($\rho_c = 0.78$) (Table 3, Figure 8a).

Table 3. Summary of the performance of PCC-KMC and PCC-KMC-CNN in 26 ShB-infected rice culm RGB images compared to visual manual annotation and ImageJ measurements.

Disease Severity Measurement	Methods	Location Shift	Scale Shift	Accuracy	Precision	Lin's Concordance Correlation Coefficient
Absolute lesion height (cm)	Visual vs. ImageJ	0.465	1.1757	0.8919	0.9259	0.8258
	Visual vs. PCC-KMC	0.3799	1.1127	0.9278	0.9398	0.8719
	Visual vs. PCC-KMC-CNN	0.5623	1.0465	0.8627	0.9026	0.7787
Proportional lesion height	Visual vs. ImageJ	0.4664	1.1704	0.8977	0.9339	0.8384
	Visual vs. PCC-KMC	0.4072	1.0792	0.9210	0.9415	0.8671
	Visual vs. PCC-KMC-CNN	0.6118	1.0311	0.8420	0.8954	0.7539
Absolute lesion area (cm ²)	ImageJ vs. PCC-KMC	−0.2149	0.9799	0.9772	0.9659	0.9439
	ImageJ vs. PCC-KMC-CNN	0.0576	1.1022	0.9936	0.9659	0.9598
	PCC-KMC vs. PCC-KMC-CNN	−0.1449	0.9876	0.9895	0.9536	0.9437
Proportional lesion area	ImageJ vs. PCC-KMC	−0.1449	0.9876	0.9895	0.9536	0.9437
	ImageJ vs. PCC-KMC-CNN	0.1483	1.1546	0.9791	0.9689	0.9486

The same trend was observed for the agreement of proportional lesion height among the different measurement methods. For the diseased lesion area (cm²), PCC-KMC-CNN was slightly more accurate ($\rho_c = 0.96$) than PCC-KMC ($\rho_c = 0.94$) upon comparing their measurement values to that of ImageJ. A similar pattern was also observed for proportional diseased area (Figure 8b). Moreover, ImageJ, PCC-KMC and PCC-KMC-CNN showed a good correlation between absolute lesion height and absolute diseased area ($r = 0.80$ to 0.86) (Table 4, Figure 9). Note that Table 4 reports agreement metrics for continuous-valued lesion measurements; therefore, classification metrics such as F1-score and AUC cannot be derived from these aggregated values and require pixel-level categorical labels, which are not part of this analysis.

Table 4. Correlation analysis of ShB lesion height and disease area of 26 ShB-infected rice culm RGB images using ImageJ, PCC-KMC and PCC-KMC-CNN.

Disease Severity Measurement	Methods	Correlation Coefficient
Absolute lesion height (cm) vs.	ImageJ	0.8045
	PCC-KMC	0.8403
Absolute diseased area (cm ²)	PCC-KMC-CNN	0.8577
Proportional lesion height vs.	ImageJ	0.7855
	PCC-KMC	0.8173
Proportional diseased area	PCC-KMC-CNN	0.8209

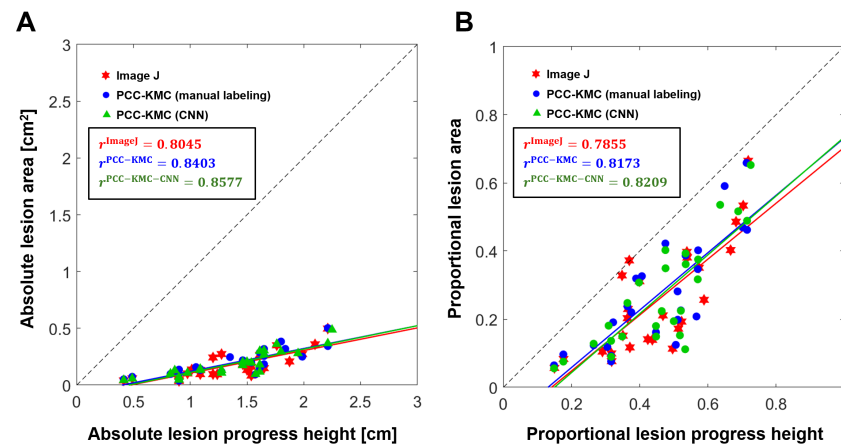


Figure 9. Correlation of ShB lesion height and symptomatic disease area. Relationship of lesion height (cm) and diseased area (cm²) in terms of (A), absolute and (B), proportional values. Red, blue, and green lines represent regression lines for ImageJ, PCC-KMC with manual labeling, and PCC-KMC-CNN, respectively.

5. Discussion

Moreover, we introduced a CNN for automatic classification in clusters produced by the PCC-KMC so that the present digital image quantification method becomes fully automatic. The performance and feasibility of PCC-KMC with manual classification and PCC-KMC-CNN were also evaluated to test their potential. A high correlation between PCC-KMC with manual labeling, PCC-KMC-CNN, and the visual-based manual annotation method was obtained. Our study has provided a new strategy to assess the severity of ShB and other plant diseases.

Furthermore, instead of using the standard Euclidean distance, we employed the Mahalanobis metric, which is unitless and scale-invariant and accounts for feature correlations. This enables more effective clustering of arbitrarily shaped disease lesions. Hence, the present PCC-KMC algorithm is more suited to segment ShB symptomatic regions on RGB images of infected rice culms and quantify ShB severity (lesion length and diseased area).

A wide variety of methods are used to describe the ShB resistance of germplasm, including disease indices [9,81], 0 to 9 scale [82] and a combination of the lesion length of three adjacent leaves [82], which poses challenges for the comparison of the results from different studies. In the field, pathogen inoculation methods such as liquid inoculum injection [83], mycelial toothpick inoculation [52] and solid inoculum injection are widely utilized, whereas under controlled conditions, the microchamber assay [9], mist-chamber [13] and detached leaf method [13,84] are used. However, previous studies reported that different evaluation methods can generate different phenotypes of ShB disease of the same genotypes [20]. For instance, two moderately resistant cultivars, such as Tetep and Teting, were found to be resistant in mist-chamber inoculations; however, under micro-chamber conditions, enhanced disease scores were observed in the two cultivars [20]. Furthermore, the environment in which the experiment is carried out [20], the morphological characteristics of rice [14,85] as well as the age of the plants being tested [9,20] are factors influencing the resistance of rice cultivars. For instance, the susceptible rice cultivar, Cypress, surprisingly showed resistance once inoculated in field conditions [9].

For polygenic disease resistance screenings such as for ShB, a wide range of susceptibility profiles among different rice cultivars can be observed [1,9,20–22]. In addition, the characteristic necrotic, water-soaked, irregularly margined ShB disease lesions that occur on the leaf sheaths and blades of rice plants [2,4,86] make it difficult to determine the exact height of the lesion in infected plants.

However, another devastating symptom caused by ShB is stem lodging, which occurs at the lower internode of rice plants [87,88] and consequently causes weakening of the rice stems. This leads to the interruption of transportation of water and nutrients through the xylem and phloem [89] and negatively affects the photosynthetic ability of the plant, leading to poor grain filling and biomass production of rice plants [90–92]. Basu and colleagues [93] reported the significant difference in lesion development between rice varieties Swarna (susceptible) and Swarnadhaan (tolerant) in terms of lesion length with the increase in time (days post inoculation). In addition, microscopic examination of tolerant and susceptible ShB-infected host tissue, wherein greater hyphal density, number of infection cushions and microsclerotia were observed in the susceptible variety, suggesting the preferential colonization of *R. solani* affecting the differential outcome of ShB disease symptoms in these varieties [93]. Since ShB symptoms tend to first appear near the stem base and then extend towards the rice canopy [2,6]. The tissue damage and obstruction of water and nutrient transportation to the upper regions of the plant affect rice production [88]. Moreover, ShB infestation was reported to reduce stem breaking resistance, leading to stem lodging [87]. Hence, these limitations emphasize the need to develop less subjective approaches capable of providing high-throughput yet accurate measurements of the quantitative ShB resistance phenotypes reflected by different rice germplasm. In addition, ShB severity and its consequent resistance phenotypes must not only be measured in terms of lesion progression but also in terms of diseased area in order to have a complete assessment of ShB resistance, which can be overcome with the use of image-based disease detection and quantification as shown in this study. In a digital image, except for boundaries, the closer pixels generally possess a higher correlation, such as pixels in the immediate neighborhood sharing similar features. In addition to color information, spatial relationships of neighboring pixels can also be of great aid in imaging segmentation, particularly for plant diseases that exhibit gradual dispersion of the diseased area or necrosis, such as ShB. Moreover, clustering considering the spatial information can yield more homogeneous clusters, less noise, and reduce the spurious blobs [94]. Numerous studies employing k-means clustering, mean-shift clustering and SLIC showed enhanced performance of image segmentation by including the spatial information in a feature space [78,94–96]. Essentially, k-means and SLIC require a non-physical parameter, the number of clusters, whereas mean-shift operates with a physical parameter, the window size or bandwidth; hence, mean-shift is often preferred for general-purpose image segmentation. However, the computational complexity of these methods is linearly proportional to the number of iterations, whereas mean-shift is relatively expensive because of its quadratic order in the number of iterations. Based on our results, PCC-KMC and PCC-MS appear to produce similar results. In fact, mean-shift clustering is a general and application-independent tool that does not require setting the number of clusters. However, its limitation is that it requires the selection of an optimal bandwidth, which is a non-trivial task and often more computationally expensive than k-means clustering and PCC-KMC. Furthermore, SLIC divides the search region into a set of small areas, which are relatively regularly shaped, it may not be efficient and accurate to segment arbitrarily shaped and scaled symptomatic regions. Hence, in this paper, we decided to use k-means clustering with spatial information to facilitate efficient processing of a high number of sample images to extract data of statistical and biological significance with ease of implementation. Acquisition of rice culm images while the plant is still planted in the pots or in the field can reduce the accuracy of image-based disease measurements due to numerous factors such as the angle of variation in image acquisition, illumination, and the distance between the sensor and the plant target. Hence, to acquire the sample images in a systematic and uniform manner, the use of destructive sampling was inevitable in this study. However, some limitations to this approach may exist since rice culms are

not as flat and thin as paper, and shadows can be cast over the margins of the plant upon scanning the stems. In addition, to prevent the sample from drying out and becoming brittle, it is recommended that the samples should be scanned right after cutting out the plant or placed in a Ziploc bag with a damp paper towel in it. Lin's concordance correlation coefficient has been used to assess the accuracy of plant disease quantification methods by comparing the estimated and actual values of disease severity measurement [15,80,97]. We observed high levels of accuracy and precision of lesion height upon comparing PCC-KMC to visual measurements and for PCC-KMC and PCC-KMC-CNN to ImageJ, which supports that the PCC-KMC method mimics the human visual measurements and the gold standard, respectively. This suggests that PCC-KMC is effective in detecting ShB in RGB images of ShB-infected rice culms and automation using this method of image segmentation is possible. With the existing ImageJ software, which requires manual-in-thresholding for every image being analyzed, the automated PCC-KMC-CNN system image will serve as a foundation for constructing a pipeline of high-throughput ShB resistance screening experiments. As for future work, we recommend the testing of PCC-KMC-CNN using a larger number of sample images with pre-determined visual-based resistance phenotype data (resistant or susceptible), which can be incorporated in the CNN and provide qualitative resistance phenotypes automatically for each image. In summary, we developed an image-based disease quantification system capable of accurate detection of ShB resistance reactions at early disease development. The performance of both PCC-KMC and PCC-KMC-CNN was confirmed with visual-based estimations of ShB disease symptoms. This method provides reliable and precise phenotypic data of ShB disease for resistant germplasm screens and breeding programs. Furthermore, the method could be easily adapted for evaluating similar diseases in other plants.

6. Conclusions

In this study, we propose an objective and automated image-processing-based methodology for quantifying the severity of rice sheath blight (ShB) and demonstrate its accuracy through comprehensive evaluations. The pipeline consists of red-green-blue (RGB) image acquisition, background removal, pre-processing, lesion segmentation, and automated classification of segmented regions. A key contribution of this work is the development of the pixel color- and coordinate-based k-means clustering (PCC-KMC) algorithm, which incorporates the Mahalanobis distance metric in a five-dimensional feature space ($R, G, B, \text{row}, \text{column}$). This design enables highly localized and robust segmentation of ShB lesions, outperforming traditional color-only clustering approaches. To classify which segmented regions correspond to true lesions, we also introduced the PCC-KMC with convolutional neural network (CNN) framework. Instead of using fixed-size image patches, which are unsuitable for irregularly shaped and variably sized lesions, we transformed each segmented region into a 1000×3 RGB histogram representation and used this compact feature vector as input to a CNN. The CNN utilized in this study successfully learned RGB histogram patterns associated with true ShB symptoms and achieved high accuracy in automatic annotation.

Extensive validation confirms the effectiveness of the proposed system. PCC-KMC with manual labeling showed high concordance with visual measurements, recording low bias and high precision for both absolute lesion height ($C_b = 0.93, r = 0.94$) and diseased area ($C_b = 0.98, r = 0.97$). The proposed PCC-KMC-CNN further demonstrated strong performance, achieving 92% classification accuracy and similarly high concordance in lesion height ($C_b = 0.86, r = 0.90$) and diseased area ($C_b = 0.99, r = 0.97$). These results indicate that the methodology offers a promising alternative to traditional, subjective, and labor-intensive visual scoring of ShB severity. Overall, the proposed PCC-KMC-CNN framework

provides a powerful and automated approach for ShB severity assessment, offering high precision, robustness, and significant potential to replace traditional visual-based methods in high-throughput phenotyping workflows.

Despite these strengths, several limitations remain. The limitations of the current work can be summarized as follows:

- Inconsistent illumination across RGB images, including shadows and brightness variations across different days, may reduce the robustness of the segmentation without appropriate normalization or pre-processing.
- The co-occurrence of multiple stresses in field environments, such as other diseases, nutrient deficiencies, and pest-induced damage, makes it challenging to isolate ShB lesions and requires further algorithmic adaptation for practical field deployment.

To address these issues, future research will explore more robust pre-processing strategies for field conditions, including 3D RGB data (RGB + depth) and additional color spaces such as hue-saturation-value (HSV) or LAB to improve segmentation stability under variable lighting. Distinguishing visually similar symptoms will likely require expanding annotation classes and building a larger, more diverse dataset. Furthermore, integration of multispectral or hyperspectral imaging has strong potential to enhance early-stage disease detection and enable large-scale field-based phenotyping applications.

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