Application of a pixel color- and coordinate-based K-means clustering algorithm and RGB color imaging for quantification of rice sheath blight infection

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Abstract
Red-green-blue (RGB) digital image-based detection is a promising alternative approach to the existing subjectivity-prone and labor-intensive plant disease quantification methods. K-means clustering (KMC) is a widely used algorithm for processing plant disease RGB images. Because the KMC algorithm clusters pixels based only on color similarity, it has limited utility for quantifying diseases that produce necrotic, coalescing, and water-soaked lesions. Here, we present a pixel color- and coordinate-based K-Means clustering (PCC-KMC) algorithm that is capable of RGB image analysis based on color (normalized RGB values) and spatial information (column and row coordinates) of pixels for quantification of rice sheath blight (ShB) disease. The performance of PCC-KMC was tested using RGB images of ShB-infected stems of the rice lines Jasmine 85, Lemont, and 301194. In contrast to KMC, which clustered randomly scattered pixels of similar color, PCC-KMC successfully segmented diseased regions into biologically-relevant clusters and thereby correctly indicated the pattern of ShB progression. PCC-KMC also enabled the quantification of disease in terms of the percentage of stem area with symptoms and the distance of disease progression from the inoculation site. The results indicate that the PCC-KMC algorithm can be used for the automated quantification of lesions caused by SbB on rice and of lesions caused by other pathogens on other plants.

Introduction
Many plant breeding programs rely on visual-based assessments of disease severity to evaluate germplasm resistance to disease (Bock et al. 2008; Hossain et al. 2016; Jia et al. 2013). The proportion of the whole plant that is diseased or disease lesion length as determined with a ruler, for example, are frequently used to assess the resistance of rice germplasm to rice sheath blight (ShB) (Hossain et al. 2016; Jia et al. 2013). These visual-based assessments of disease evaluation are time-consuming, cost-ineffective, and vulnerable to evaluator subjectivity (Bock et al. 2008; Jia et al. 2007; Jia et al. 2003; Lee et al. 2002; Poland and Nelson 2011).

The current report describes a non-visual approach for assessing ShB resistance in rice germplasm. ShB, which is caused by Rhizoctonia solani, can drastically reduce rice production (Banniza and Holderness 2001; García et al. 2006). Given the absence of complete ShB resistance in rice germplasm (Hossain et al. 2014; Jia et al. 2007), an objective, precise, and reliable quantification tool is needed to screen large germplasm collections for ShB resistance.

The quantification of plant diseases has recently been improved by the development of various imaging systems (Barbedo 2013; Mahlein 2016). These imaging technologies have substantial potential to provide disease severity data with enhanced precision and reproducibility
and with reduced potential for the errors caused by visual assessment (Rousseau et al. 2013). Red-Green-Blue (RGB), which is one of the many techniques used for digital image-based quantification of disease severity, generally consists of three major steps: (i) pre-processing (background removal), (ii) segmentation (the clustering of pixels based on color), and (iii) feature extraction and identification of the object of interest (Gupta et al. 2017; Khirade and Patil 2015; Kuruvilla et al. 2016). In this study, we focused on optimizing the K-means clustering (KMC) algorithm, which is one of the most commonly used algorithms for image segmentation in image-based disease detection (Al-Hiary et al. 2011; Al Bashish et al. 2011). Traditional KMC segments RGB images into a given number (“K” number) of clusters based on the closeness of the RGB values of pixels. This approach is useful when there is a one-to-one correspondence between specific RGB values and disease symptoms. However, variances of pixel coordinates (column and row index information) are expected to be large for KMC, resulting in weak spatial correlations among pixels even within the same cluster. To reduce this limitation of KMC, we developed a Pixel Color and Coordinate-based K-Means Clustering (PCC-KMC) algorithm that takes into account both color and spatial attributes. By capturing biologically relevant clusters, PCC-KMC enables a robust and automated quantification of the severity of ShB as well as of other diseases that produce similar lesions.

Materials and Methods

Preparation of ShB-infected plant samples

Plant materials used in this study

The rice cultivars/accessions Jasmine 85 and 301194 (both of which are moderately resistant to ShB) and Lemont (which is highly susceptible to ShB) were obtained from the Genetic Stocks Oryza Collection, USDA-ARS, Dale Bumpers National Rice Research Center, Stuttgart, Arkansas (Eizenga et al. 2014). De-husked rice seeds were surface-sterilized with a 10% Chlorox solution, germinated on ½ Murashige and Skoog (1/2 MS) medium, and transplanted into plastic pots (15 cm in diameter) filled with ProMix BX™ soil. The seedlings were grown in a growth chamber (26°C on day and 20°C at night, 12 hr light/dark cycle, and 80% relative humidity) until they were ready for inoculation.

Inoculation of ShB pathogen and disease induction in rice plants

Rice seedlings with four to five leaves were inoculated with isolate B2 of the ShB pathogen R. solani (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). A micro-chamber method (Jia et al. 2007) was used to provide optimum conditions for ShB development, and inoculated seedlings were placed in growth chambers at 28-30°C with a relative humidity of ~90%. At 7 to 10 days post inoculation, the infected culm (stem) portion of the rice plant were excised to acquire RGB digital images.

Acquisition of RGB digital images of ShB-infected rice stems

All RGB digital images of ShB-infected rice stems were obtained using a flatbed scanner (HP Scanjet G4050) under controlled conditions to ensure uniformity of the distance of image acquisition. To reduce potential background noise in the RGB image, a monochromatic, matt black paper was used as background for the plant samples placed on the scanner. Images with 600 pixels
per inch resolution were saved in PNG format. All image analyses were performed using the image toolbox in the MATLAB® programming language.

**Image analyses of ShB-infected rice stems**

*Pre-processing of acquired RGB images*

Using the schematic layout of the background removal algorithms (Fig. 1A), we isolated the image of our interest (Jasmine 85 stem) from the whole RGB image. To extract green-dominant pixels (non-diseased region), initial screening was performed by using the following simple threshold criterion:

$$I_G(r, c) > I_R(r, c) \text{ and } I_G(r, c) > I_B(r, c)$$

where \(I_R, I_G, \) and \(I_B\) represent red, green, and blue values of a given input RGB digital image, respectively, while \(r\) and \(c\) indicate row and column pixel index, respectively. By labeling pixels which meet the above condition as 1, otherwise 0, one can build two binary images or masks to decompose the input RGB image into non-diseased and potentially diseased regions. Although the thresholding method is extremely fast in segmenting RGB images, its limitation is that shaded regions typically distributed around borders of rice ShB lesions may not be accurately identified. Nevertheless, the purpose of using the initial screening is to roughly filter out healthy regions for certain first so that subsequent clustering process would be faster and more accurate to distinguish diseased regions.

*Segmentation of RGB images*

An image of an ShB-infected stem of a Lemont seedling was used to compare the segmentation performance of KMC and PCC-KMC. Given a set of \(L\) number of observations \((y_1, y_2, ..., y_L)\), each observation includes RGB color values at a \(r^{th}\) and \(c^{th}\) pixel, i.e., \(y_i = \{I_R(r, c), I_G(r, c), I_B(r, c)\}\), where \(L\) is equal to the total number of pixels of the possibly symptomatic regions which were labeled by 0 from the initial screening. Then, KMC partitions \(L\) observations into a given number \(K (\leq L)\) sets, denoted as \(S = \{S_1, S_2, ..., S_K\}\) in such a way of minimizing the total sum of squared distances between observations and centroids of the clusters, such as

$$\arg \min_S \sum_{i=1}^{K} \sum_{x \in S_i} \|x - \mu_i\|^2$$

where \(\mu_i\) is the mean of points \(S_i\). In the case of PCC-KMC, for a given a set of observations \((x_1, x_2, ..., x_L)\), each observation is a 5-dimensional real vector composed of normalized RGB color values and normalized row and column pixel indices as follows:

$$x_i = \left\{ \frac{I_R(r, c)}{\max(I_R)}, \frac{I_G(r, c)}{\max(I_G)}, \frac{I_B(r, c)}{\max(I_B)}, \frac{r}{\max(r)}, \frac{c}{\max(c)} \right\}$$

The elbow method was used to determine the optimal \(K\) of both KMC and PCC-KMC (Bholowalia and Kumar 2014).
Quantification of disease in plant sample images

All clusters were identified into one of three classes: (1) diseased, (2) dried, or (3) healthy. Note that the three classes reflect on the representative patterns observed in ShB-infected rice plants. Specifically, for the identification step, RGB color spectrums were created for all clusters. Among these clusters, three clusters were manually selected to represent the diseased, dried and healthy classes and their RGB color spectrums were set to be references of these three classes. Finally, through evaluation of the degree of color-spectrum-correlation between three references and each cluster, we determined one of three classes for each cluster by choosing the highest correlation value.

To quantify disease progression and severity using PCC-KMC, we analyzed the RGB images of ShB-infected accession 301194 seedlings were analyzed. Disease lesion length, i.e., the distance from the base of the plant to the farthest disease lesion (Jia et al. 2007), was calculated as the percentage of the total length of the stem. In addition, the disease lesion area was calculated as a percentage of the total area of the stem.

Results

The acquired RGB images of the stem of Jasmine 85 (Fig. 1B) were pre-processed using two key parameters associated with the performance of the background remover: (i) the edge detection type with a sensitivity parameter (Fig. 1C) and (ii) the element type and size for dilation and erosion (Fig. 1D). As a result, the Jasmine 85 stem was isolated from the background (Fig. 1E).

Fig. 1. Steps in the pre-processing of red-blue-green (RGB) images to isolate the plant sample of interest. A, Schematic diagram of the background-removal steps used in this study. B, Input RGB digital image of the stem of a sheath blight-infected rice plant (cultivar Jasmine 85). C, Binary images after using the edge detection with dilation. D, Binary images after filling and erosion. E, Masked RGB digital image after C and D were completed.

The well-masked RGB image of the Jasmine 85 stem was then subjected to a simple thresholding method, which decomposed the plant sample of interest into regions without ShB disease symptoms (green regions) and with ShB disease symptoms (brown to black lesions) (Fig. 2A). For this image, we used five clusters (k = 5), since this was the optimal K value calculated.
by the elbow method and this value was within 90% convergence for both KMC and PCC-KMC (Fig. 2B). Because KMC does not consider any spatial information for clustering, the resultant clusters were expected to be globally distributed in the plant sample image in terms of color similarity. With PCC-KMC, in contrast, clustered regions indicate color similarity but also spatial proximity (Fig. 2C). As a result, the diseased regions segmented by KMC resulted in clusters containing pixels based on color similarity (RGB values) regardless of the location of pixels in the image (Fig. 2D), whereas the diseased regions segmented by PCC-KMC resulted in clusters containing pixels based on both color similarity (RGB values) and spatial proximity (Fig. 2E).

Fig. 2. Image segmentation of the stem of a sheath blight (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 dominant) from those with disease symptoms. B, Result of the use of the elbow method to obtain optimal number of K for both KMC and PCC-KMC. C, Working principle for image segmentation used in KMC and PCC-KMC algorithms. Pattern of pixel clustering using D, KMC and E, PCC-KMC.
When we tested PCC-KMC on another RGB image of a Lemont stem that had the most severe disease lesions among our samples, the image was successfully partitioned into non-diseased (white) and diseased (light and dark gray) regions (Fig. 3).

**Fig. 3.** Pre- and post- PCC-KMC segmentation of an image of the stem of a sheath blight (ShB)-infected rice plant (cultivar Lemont).

After performing segmentation using PCC-KMC (Fig. 4A), we then quantified ShB symptoms using an RGB image of rice accession 301194. In this case, the percentage of disease progression with respect to the total length of the stem was 24.68% (Fig. 4B), and the diseased area with respect to the total plant stem area was 1.4% (Fig. 4C). In addition, the diseased area with respect to the stem area up to the farthest lesion height was 5.8% (Fig. 4D).

**Fig. 4.** Quantification of rice sheath blight (ShB) using PCC-KMC. A, Original red-green-blue images and B, Post-PCC-KMC segmentation images of cultivar 301194. B, Percentage of disease progression relative to the total height of the plant sample. C, Percentage of diseased area relative to the total area of the plant sample. D, Percentage of diseased area relative to the area of the plant sample up to the top of the lesion.

**Discussion**

Precise quantification of ShB is challenging due to characteristic necrotic, water-soaked, irregular-margined disease lesions occurring on leaf sheaths and blades of rice plants (Banniza and
Holderness 2001; Ogoshi 1987; Yellareddygari et al. 2014). In this study, we developed an RGB image-based disease quantification method that uses the PCC-KMC algorithm, which allows automated and objective quantification of ShB. PCC-KMC allows the capturing of disease progression by taking into account both the color similarity and physical proximity of pixels. In contrast to the traditional KMC, which only takes into account the color similarity of randomly scattered pixels on the plant sample, PCC-KMC clusters pixels based on both color and spatial proximity and therefore provides data with greater biological relevance. PCC-KMC will be useful in assessing the levels of ShB resistance in rice germplasms (Li et al. 1995; Pinson et al. 2005) and will also serve as a foundation for the development of automated detection of disease lesions that are similar to those produced by ShB on rice.

Because ShB symptoms tend to first appear near the stem base and then extend towards the rice canopy (Hashiba and Kobayashi 1996; Ogoshi 1987), measurement of the vertical distance that disease lesions have grown has often been used to assess ShB resistance (Jia et al. 2013). The lesions on lower internodes of rice stems, however, cause tissue damage and stem lodging and are therefore especially important. The resulting obstruction of water and nutrient transportation to the upper regions of the plant decreases rice production (Hoshikawa and Wang 1990). Furthermore, some cultivars with moderate ShB resistance have small disease lesions that are located far from the stem base (Fig. 4A). For these reasons, a reliable assessment of ShB requires the assessment of both vertical disease progression and the area of the diseased tissue.

Aside from the K-means clustering approach utilized in this study, hierarchical clustering or mean shift clustering techniques (Comaniciu et al. 2002) may be used as an alternative approach in RGB image processing for plant disease quantification. Both of these methods do not require the determination of optimal cluster numbers. However, hierarchical clustering requires high computational cost and time, whereas means shift clustering requires another parameter, such as bandwidth or window size, to be determined.

Since each axis of the five-dimensional space represents different physical meaning, viz., colors and coordinates, distances between pairs of arbitrary data may not be invariant under translation or rotation based on the origin of the five-dimensional space. In this study, we utilized normalized Euclidean distance was used to measure the closeness of data and show differences in terms of scales. However, for a more stringent condition of the distance measurement in five-dimensional color and metric space, we recommend the use of the Mahalanobis distance, which considers the scaling difference in each axis incorporated into the covariance matrix (Melnykov et al. 2014).

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